Intrahippocampal clodronate administration alters the brain inflammatory response to systemic LPS in mice

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POSTERS

Poster abstracts

Poster topic 01
Cell migration

T01-01A

PAR-3 and Syndecan-4 are involved in astrocyte adhesion induced by neuronal Thy-1

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We have shown previously that astrocyte adhesion is induced by the interaction of the neuronal protein Thy-1 with avb3 integrin and Syndecan-4 in astrocytes. However, the signaling mechanisms triggered downstream of these astrocyte receptors remain unknown. PAR-3 is an important adaptor protein that participates in the polarization of many cell types by forming a complex with PAR-6/aPKC; however, PAR-3 is not part of this complex during astrocyte polarization. Therefore, PAR-3 function in astrocyte adhesion remains to be determined. Here, we evaluated the participation of both Syndecan-4 and PAR-3 in astrocyte adhesion induced by Thy-1.

Rat DITNC-1 astrocytes were transfected with siRNA against PAR-3, Syndecan-4 or siRNA control, treated with Thy-1-Fc or TRAIL-R2-Fc as a control and then assayed for wound-healing and focal adhesion formation. For focal adhesion analysis the cells were stained with anti-vinculin. Focal adhesion number and morphology were evaluated by confocal imaging.

Our results show in DITNC-1 cells transfected with siRNA for PAR-3 or Syndecan-4 and stimulated with Thy-1 that wound-closure was decreased compared with siRNA control-transfected cells. For cells transfected with PAR-3 or Syndecan-4 siRNA larger focal adhesions than control cells were observed, implicating PAR-3 and Syndecan-4 in astrocyte adhesion induced by Thy-1. Whether these proteins regulate focal adhesion disassembly will be addressed in future studies.

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T01-02A

Two-Photon polymerized microstructures for guiding cell growth in neuron and astrocyte co-cultures

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Question: Nervous system is composed of organized anatomical structures where neural cells have oriented growth and polarized morphology. This kind of orientation is needed for obtaining functional signal transduction and processing system that allows us to make decisions, carry them out, get feedback and learn. On nervous system deficits, the regeneration of this system is very limited. Tissue engineering might in future be able to enhance regeneration and retrieve lost functionality. In tissue engineering, one strategy is to give cells a scaffold, an artificial extra cellular matrix, which could support regeneration. Cell growth guidance is desirable for obtaining organized structures, which could enhance functional neural circuit regeneration. Guidance can be obtained using physical and chemical cues, which provide a guiding environment leading to cell orientation.

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The aim of this work was to study the effect of topographical cues on growth of neural cells.

Methods: Microstructures were manufactured using two-photon polymerization. Microstructure quality was analyzed using scanning electron microscopy. Cell cultures were prepared using human pluripotent stem cell derived neural cells, neurons and astrocytes. Cell phenotype was analyzed using immunocytochemical staining with cell type specific markers.

Results: Cell viability was not compromised by microstructures. Cells grew on the microstructures and some degree of guidance was seen especially with neurons.

Conclusions: In terms of growth, the human neural cells seem to have different matrix demands compared with the published literature about their rodent counter parts. Nevertheless, it seems that when it comes to topographical guidance cues in culture, neural cells grow on the microstructures despite their origin.

T01-03A

G_s protein coupled receptor signalling strongly represses PI3Kγ -driven microglial migration

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The PI3Kγ, a member of the class IB of the PI3K family, has been shown to regulate various cellular functions especially in cells of the immune system. Microglia represent the major effectors of the innate immune response in CNS. During the course of inflammatory processes the dormant glial cells become activated. The activation of microglia involves a complex cascade of changes including expression of various receptors and morphological changes, secretion of pro - and anti -inflammatory cytokines, generation of ROS (reactive oxygen species), nitric oxide, neurotrophic factors and changes in phagocytotic and migratory activities of the cells. Our former studies indicate a lipid kinase independent involvement of PI3Kγ in the phagocytosis of microglial cells. It could be shown in microglial cells that the PI3Kγ-inducing G_i protein coupled signalling and the competing G_s protein coupled signalling co-ordinately regulate each other. Phosphodiesterases (PDEs) bridge both pathways in that they catalyse degradation of cAMP, the central G_s protein coupled signalling second messenger. The focus therefore is on the influence of classical lipid kinase activity and lipid kinase independent function of PI3Kγ on microglial migration. Ongoing studies demonstrated that wild type microglia show a significantly higher migratory activity in comparison to PI3Kγ knockout and PI3Kγ knockin (kinase dead) cells, implicating the lipid kinase activity of PI3Kγ in microglial migration. Further investigations could show that independent of the kinase activity a manipulation of G_s protein coupled signalling by using either stimulators like Forskolin and Norepinephrine or PDE inhibitors like Rolipram, Cilostamid and IBMX significantly suppresses the PI3Kγ -driven migration. This suppression could be rescued by an inhibition of the G_s protein coupled signalling by H89 (Protein kinase A inhibitor). These findings throw new light on the expanding role of PI3Kγ in the regulation of microglial migration, i.e., its scaffold function in addition to PIP_3 generation by lipid kinase activity. In summary the study explored PI3Kγ and G_i/G_s signalling as mediators of microglial mobility; alternate protein functions increase complexity making further investigation along these lines important for better understanding microglial functions in immunity and disease/inflammation.

Acknowledgement: This work is funded by the DFG (Deutsche Forschungsgemeinschaft) and the University Hospital Jena.[1, 2]

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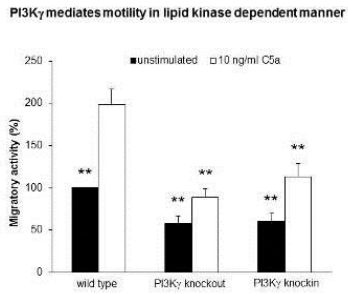
Image



(PI3Kγ mediates migration of primary microglia in a lipid kinase dependent manner. Migration assay was performed for 2 h with wild type, PI3Kγ knockout and PI3Kγ knockin microglia. Cells were allowed to migrate to 10 ng/ml C5a. 100 000 cells/insert. Significant to wild type C5a (*); ** $p \leq 0.01$; n=4)

T01-04A

Water fluxes and aquaporins in migrating oligodendrocyte progenitor cells

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In the past 15 years evidence emerged that, in addition to changes in actin polymerization, ion and water fluxes are essential for epithelial cell migration (Schwab *et al.*, 2007). The postulated mechanism is that ion transporters and ion channels create a locally restricted increase of osmotic pressure near the leading edge of the cell which is followed by a passive influx of water through aquaporin (AQP) water channels (Papadopoulos *et al.*, 2008).

We have previously applied Scanning Ion Conductance Microscopy (SICM), a contact-free scanning probe technique that allows obtaining three-dimensional images of single living cultured cells, to monitor migration of bipolar oligodendrocyte progenitor cells (OPCs). Our observations presented for the first time direct evidence that local swellings indeed occur at the leading edge in migrating OPCs indicating a potential role of water fluxes across the membrane during migration of this particular cell type (Happel *et al.*, 2013).

Here, we investigated, whether particular aquaporins could be involved in the migration of OPCs by performing immunocytochemical investigations using antibodies against AQPs 3, 4, 5 and 8, which have been reported to be expressed in the brain but have to our knowledge not been studied in OPCs. First results showed a highly polarized expression of AQP3 in OPCs whereas the antibodies against the other aquaporins showed no specific staining in OPCs. We are now resolving the different saltatory phases of OPC migration with an improved SICM with three times higher temporal resolution in more detail. The improved instrument already allowed us to study sub cellular volume and velocity changes during the migration of dorsal root ganglion neurons(Gesper *et al.*, 2015). In combination with the immunocytochemical investigations we want to elucidate, whether AQP3 dynamically polarizes to the leading edge of accelerating cells.

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T01-01B

Crosstalk between early fate determinant and chemotropism controls collective glial migration

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Collective cell migration is a key process during development as it allows the organization of complex tissues such as the nervous system, which comprises of a vast and complex cellular network that is essential for its proper functioning. To maintain the circuitry, neurons and glia migrate collectively from their birthplace to a distant site. Defects in this process lead to severe pathologies including mental retardation. Collective cell migration is an extremely dynamic process that needs cell interaction, however, its precise behavior calls for an autonomous cues being at work as well. While the role of molecules controlling cell interactions has been extensively investigated, the signaling cascades that trigger chemotropism are not understood. To this purpose, I have analyzed the impact of an early transcription factor in the process. The glial chain in a developing *Drosophila* wing provides an excellent tool to study the molecular pathway underlying collective migration.

As the main project of my thesis, I have asked whether the transiently expressed Gcm transcription factor, which triggers the fate choice between glia and neurons, also controls collective migration. I demonstrate that Gcm directly affects migration in a dosage dependent manner by inducing the expression of Frazzled, a chemoattractant membrane Netrin receptor.

Netrins are a class of secreted proteins and serve as guidance cues for navigating axons. They act as ligands with the help of the attractant receptor Frazzled and the repulsive receptor Unc5.

Interestingly, I have found that Frazzled and Unc5 control glial migration: Frazzled strongly lowers migration efficiency when downregulated in glia and enhances it when overexpressed. In contrast, Unc5 slows down migration when overexpressed.

I have followed Frazzled more closely and confirmed its cell autonomous role by the complete rescue of the mutant migratory phenotype upon reexpressing Frazzled specifically in glial cells.

Further, I have validated that Frazzled is a direct Gcm target. Finally, Frazzled overexpression rescues the Gcm loss of function phenotype. These data indicate that Frazzled is epistatic to Gcm.

My data demonstrate for the first time the direct role of a fate determinant on a late and collective behavior. Hence, the integration of autonomous (Gcm) and regulatory (Netrin) pathways ensures that glial migration occurs in a timely and efficient manner.

T01-02B

Myelin proteolipid protein mediates the association of alpha V integrin and the AMPA glutamate receptor *in vivo* and regulates glutamate-induced migration of oligodendrocyte progenitor cells through GluR2 internalization

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Question: Earlier studies on cultured oligodendrocyte progenitor cells (OPCs) demonstrated that stimulation of AMPA glutamate receptors induces the formation of a signaling complex that includes subunits of the AMPA receptor, integrins, calcium-binding proteins, and, surprisingly, the myelin proteolipid protein (PLP). AMPA also causes increased OPC migration in culture. The current studies focused on formation of the PLP-av integrin-AMPA receptor complex *in vivo,* and the impact of complex formation on OPC migration in the developing brain.

Methods: Immunohistochemical and western blot analysis was performed on postnatal murine cerebellum to examine expression of PLP, av integrin and GluR2. PLPnull, GluR2null, and av integrin heterozygous mice were crossed to PLP-eGFP mice and used to make cerebellar slice cultures for live imaging of OPC migration. Live imaging of OPC migration in slices was conducted at baseline and in the presence of 2μM AMPA. Chemotaxis and AMPA receptor internalization assays were performed on cultured OPCs.

Results: We found that association of the AMPA glutamate receptor and av integrin was decreased in cerebellum of PLPnull mice. Live imaging studies of OPC migration in *ex vivo* PLPnull cerebellar slices demonstrated altered OPC migratory responses to AMPA. OPC migration was also reduced in slices from GluR2 null and av integrin heterozygous mice. Chemotaxis assays of purified OPCs revealed that AMPA stimulation was neither attractive nor repulsive, but it clearly increased the migration rate of wild type but not PLPnull OPCs. AMPA receptor stimulation of OPCs caused increased internalization of the GluR2 subunit of the AMPA receptor in wild type but not PLPnull OPCs.

Conclusions: Together these studies demonstrate that PLP serves as an important scaffold signaling protein *in vivo*, increasing the association of av integrins with AMPA receptors, which is required for increased OPC migration in response to glutamate signaling. These results have important implications for OPC responses when levels of glutamate are high in the extracellular space such as following demyelination.

These studies were supported by NIH NS25304.

T01-03B

Role of ERK and Rho associated protein kinase (ROCK) signalling in PDGF-A induced Oligodendrocyte progenitor cells migration and cytoskeleton reorganization

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Oligodendrocyte progenitor cells (OPCs) migrate from the subventricular zone towards the white matter where they differentiate into myelin-forming oligodendrocytes. They myelinate neuronal axons and thereby facilitate saltatory conduction of action potentials and provide trophic support for neurons. Thus, Migration of OPCs is essential for proper myelination of neurons. Platelet derived Growth Factor-A (PDGF-A) is one of the growth factor which promotes OPCs migration. Several intracellular signalling pathways are activated by the influence of growth factors and chemokines during OPCs migration. PDGF-A promotes OPC migration through the activation of Extracellular signal-regulated kinase (ERK). Rho associated protein kinase (ROCK) is a serine/threonine protein kinase with a multi-variate function including the regulation of cellular contraction, motility, morphology, polarity, cell division, and gene expression. The present study was carried out to understand the crosstalk of the ROCK pathway with the ERK pathway in PDGF-A induced OPC migration and to investigate the role of ROCK in regulation of pERK1/2 level and actin cytoskeleton reorganization which leads to OPC migration. OPCs were treated with Y-27632, a ROCK inhibitor, and U0126, an upstream MAPK kinase inhibitor (MEK), alone or in combination with one another. OPC migration and pERK1/2 levels were determined by agarose drop assay and immunoblotting respectively. ROCK inhibitor treatment alters the PDGF-A induced migration and pERK1/2 level in OPCs. In addition to role of ERK in migration, We further studied the interaction of activated ERK with other focal adhesion proteins in presence or absence of PDGF-A and MEK inhibitor U0126.

Poster topic 02
Cell proliferation, lineages and differentiation

T02-01A

Dissecting the role of *Etv5* in oligodendrocyte development in the neocortex

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The neocortex is an excellent example of an organ that relies on dynamic changes in the temporal identities of progenitors to consecutively generate distinct populations of neuronal and glial cells. Oligodendrocytes are a type of glial cell that are the last-born cells in the neocortex. How cortical progenitors switch their fate to begin making oligodendrocyte precursor cells (OPCs) in the postmitotic cortex is not well understood. We and others found that RAS/ERK overactivity in cortical progenitors promotes an OPC fate at the expense of a neuronal identity. This fate switch is mediated by altered transcription factor expression; *Neurog2* is turned off while *Ascl1/Etv5* are turned on. Here we investigated the role of *Etv5* in OPC fate specification. In gain-of-function assays, we found that *Etv5* promotes proliferation and the acquisition of a glioblast fate. However, *Etv5*-overexpressing glioblasts do not convert to an OPC fate unless RAS/ERK signaling is elevated. Conversely, in *Etv5* hypomorphic mutants, we found that fewer glioblasts and OPCs are generated. We have therefore identified *Etv5* as a key regulator of a glioblast/OPC fate in the neocortex.

T02-02A

Evidence for oligodendrocyte dedifferentiation and subsequent formation of astrocytes after acute brain injury

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Acute brain injuries lead to glial scar formation. Quiescent mature astrocytes get activated and proliferate in response to brain injuries. In recent years, studies suggested that a population of oligodendrocyte precursor cells (OPCs) could generate astrocytes after acute cortical injuries, though the contribution of OPCs to astrogliosis varied with animal and injury models. Here, we report that not only OPCs but also oligodendrocytes take part in the astrogliosis. Using NG2-CreERT2 knock-in mice we selectively induced a genetic label into OPCs and their putative progeny. Subsequently, we observed that acute brain injuries induced more astrocytes with this genetic marker when the pool of labeled oligodendrocytes was increased. Immunohistochemical and *in vivo* two-photon laser scanning microscopy analyses showed that a subpopulation of activated oligodendrocytes displayed astroglial transgenic GFAP promoter activity after a variety of acute cortical injuries. We termed these cells "AO cells" because of their astro- and oligodendroglial properties. Targeting exclusively AO cells with a split-Cre mouse model showed a broader, but glia-restricted differentiation potential of AO cells giving rise to astrocytes, OPCs and oligodendrocytes. The differentiation of AO cells appeared to be dependent on local cues: AO cell-derived astrocytes were more frequently found in the superficial cortical layers I-III, while AO cell-derived oligodendrocytes were preferentially located in layers V-VI near the corpus callosum. Using qRT-PCR, we found higher expression levels of glial differentiation factors such as bone morphogenetic protein 4 (BMP4), interleukin 6 (IL-6), ciliary neurotrophic factor (CNTF) and leukemia inhibitory factor (LIF) at the lesion site. In addition, we could promote the formation of AO cells by intra-cortical injection of IL-6, while LIF appeared to inhibit the dedifferentiation of oligodendrocytes. In conclusion, our data suggest that not only OPCs but rather the whole oligodendroglial lineage including oligodendrocytes has the ability to generate astrocytes after acute cortical injuries dependent on local cues.

T02-03A

Clonal distribution pattern reveals glial heterogeneity

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During embryonic life, the differentiation of all central nervous system (CNS) cells follows a sequential paradigm. Thus, neurons are generated first, followed by astrocytes and then by oligodendrocytes, all happening in partially overlapping waves. If we focus on glial cells, they are classically classified into astrocytes, oligodendrocytes, and NG2 cells. During the developmental many genetic and environmental factors are involved in the differentiation of the precursor cells, including spatial patterning cues along the rostrocaudal or dorsoventral axes of the CNS. Neural precursors cells are clustered in domains and those that are part of the same domain have mixed neurogenic and gliogenic potencies. During the last years, many works have focused on the relationships between cell identity at early stages of CNS with their final fate and phenotype, including morphology, location and function. Recently, development of new methods to study clonal cell identities has allowed establishing relationships between glial lineages and cell heterogeneity. Among them, one of the most innovative tools developed during the last years is the *Star Track* method. This technique is a genetic engineering tool that allows analyzing both astrocyte and NG2 lineage through the combinatorial expression of fluorescent proteins from their original progenitor cells to their final differentiated stages. The specifically labeled progeny can be accurately used to find relationships between the ontogenetic origin of different glial cell types with their final heterogeneous morphologies and functions. Analyses of clonal dispersion in the adult cortex revealed specific clonal distribution patterns and the existence of clonal arrangements in specific domains.

T02-04A

Origin and generation of different astroglial phenotypes in the cerebellum

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The anatomical and functional complexity of the cerebellum is reflected by a remarkable heterogeneity of neuronal and astroglial subsets, with highly distinctive morphological and spatial features. While mechanisms of neuronal diversification have been partly clarified, astrogliogenesis remains poorly explored. Here we addressed the origin(s) and the genesis of the repertoire of astroglial types. Firstly, in order to study the lineage relationships among different astroglial phenotypes, we performed in vivo clonal analysis using Star Track plasmids and Confetti mice. We found clones containing both cortical protoplasmic and white matter (WM) fibrous astrocytes. Clone composition indicates the existence of three major embryonic progenitor types producing either granular layer astroglia, or WM astrocytes, or a mixed progeny composed of Bergmann glia (BG) and granular layer (GL) astrocytes. Postnatally, these latter clones are likely to derive from radial progenitors located in the PCL dividing in situ to generate both BG and GL astroglia. Furthermore, WM astroglial clones appear smaller compared to those in the cortical layers. Double-thymidine analogues and birthdating analysis showed that these differences in clone size were associated with distinct proliferative behavior of astrocyte precursors in distinct cerebellar layers. In particular, those of the PCL (i.e. immature BG) revealed the highest division rate. Additionally, they displayed different proliferative rhythms in crowns and fissures during early postnatal development, suggesting a contribution to cerebellar foliation. We then investigated by means of homo/heterochronic transplants the role of environmental instructive cues, revealing that specific astroglial phenotypes are instructed by temporally defined extrinsic signals. In conclusion, our study reveals that cerebellar astrogliogenesis occurs from distinct embryonic progenitors according to a well-defined spatiotemporal pattern.

T02-05A

Regulatory T cells enhance oligodendrocyte differentiation *in vitro*

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One of the greatest unmet clinical needs in demyelinating diseases such as Multiple Sclerosis (MS) is to provide therapies that actively enhance the process of myelin regeneration (remyelination) in the central nervous system (CNS), which would lead to recovery of neurological function. Therefore uncovering the mechanisms that govern remyelination will prove highly beneficial to patients. Oligodendrocytes and oligodendrocyte progenitor cells (OPCs) are the myelinating cells of the CNS and therefore play a central role in remyelination. It has been shown that cells of the adaptive immune system, particularly $CD4^+$ T cells, promote remyelination *in vivo* however the contributions of distinct T cell subsets are not known. Based on our observations that regulatory T cell (Treg) depletion impairs remyelination *in vivo*, we hypothesized that Treg enhance remyelination through a direct effect on oligodendrocyte lineage cells.

To test this hypothesis, murine Tregs were first differentiated *in vitro*, in parallel with non-polarized $CD4^+$ T cell controls, and media from these cultures were harvested. The effects of these conditioned media on OPC proliferation, survival and differentiation were then investigated in an *in vitro* model of murine mixed glial cells. To elucidate the underlying signaling mechanisms, cells were stimulated with Treg-conditioned media in the presence or absence of specific neutralizing antibodies.

Soluble factors produced by Treg significantly enhanced OPC differentiation without having an effect on OPC proliferation, survival or overall oligodendroglial numbers. We could further identify a soluble factor produced by Treg that mediates this differentiation-enhancing effect.

These studies suggest a novel beneficial role of Treg in remyelination through the enhancement of OPC differentiation. Recently it was uncovered that Treg have an intrinsic repair function in muscle regeneration that is distinct from immune modulation. A similar intrinsic function in regeneration of myelin by driving OPC differentiation is likely. Molecular pathways through which the candidate Treg-secreted protein induces OPC differentiation are currently under investigation.

T02-06A

Peptidylarginine deiminases as regulators of the epigenetic state of oligodendrocyte precursor cells

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Epigenetics changes at neural cells have been suggested to play an important role in the development of multiple sclerosis (MS). For example, increased histone H3 citrullination, was reported in the brain of MS patients, possibly mediated by peptdidylarginine deiminase 4 (PADI4). Citrullination is the conversion of the positively charged peptidyl-arginine to the neutral peptidyl-citrulline. Citrullination of histones leads to local chromatin decondensation and has recently shown that it regulates pluripotency. Hipercitrullination also occurs on cytoplasmatic proteins such as myelin basic protein (MBP), one of the components of the myelin sheath. While most studies have been focused on PADI role in the citrullination of cytoplasmatic proteins in disease, little is known on the epigenetic role of PADIs on oligodendrocyte precursor cells (OPCs) lineage progression both in health and in multiple sclerosis. We have found that PADI2 and PADI3, but not PADI4 are expressed in OPCs. Furthermore, PADI2 is located both in the nucleus and cytoplasm of OPCs and is upregulated during differentiation from OPCs to mature OLs. In contrast, PADI3 expression levels are downregulated upon OPC differentiation. Interestingly, our data indicates that inhibition of PADIs leads to an arrest of OPCs in an undifferentiated, immature state. As such, we hypothesize that PADI2/3 might be involved in the pathological process of MS, not only by mediating citrullination of MBP, but also by regulating the epigenetic state of OPCs and the remyelination process.

T02-07A

Clathrin-mediated endocytosis is critical for Schwann cell myelination

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Mutations in dynamin 2 (*DNM2*) lead to dominant intermediate Charcot-Marie-Tooth neuropathy type B (DI-CMT1B) and dominant centronuclear myopathy (CNM). DNM2 is a GTPase involved in membrane fission, clathrin-mediated endocytosis (CME), vesicular trafficking from and to the trans-Golgi network, centrosome cohesion and actin and microtubule dynamics. DNM2 is essential for Schwann cell myelination, even though the molecular mechanism is only partially clear due to DNM2 pleiotropy. Therefore this work is aimed at investigate specifically the role of CME in Schwann cells myelination through deletion of the adaptor protein 2 (AP-2).

T02-08A

A novel automated dissociation procedure allows efficient immunomagnetic isolation of astrocytes, oligodendrocytes, and neurons from adult rodent brain tissue

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Careful tissue dissociation and preparation of single-cell suspensions with high cell viability and a minimum of cell debris is the prerequisite for cellular analysis, cell culture, and cell separation.

In neurobiology, single cell suspensions are often prepared from embryonic or neonatal rodent neural tissue as neural cells are not fully integrated in the neural network and tissue dissociation is relatively easy. In contrast, adult brain dissociation is very demanding and requires sophisticated mechanical and enzymatic treatment to degrade the extracellular matrix and successfully disaggregate the tightly connected neural cells.

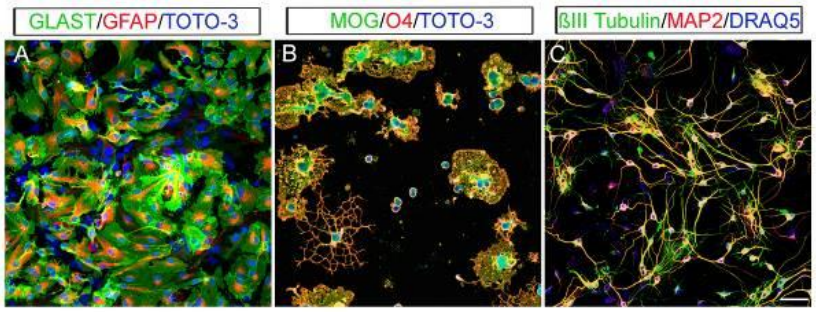
We have developed a new automated method for gentle dissociation of adult rodent brain tissue by combining mechanical dissociation using the gentleMACS Octo Dissociator (Miltenyi Biotec) with an optimized enzymatic treatment. The dissociation is followed by a novel protocol for removal of debris and erythrocytes, which is crucial for subsequent successful cell isolation. The process was optimized to increase the number of viable cells and yields 3-5x10E6 living cells per mouse brain.

Subsequently, astrocytes, oligodendrocytes, or neurons were isolated by magnetic cell sorting (MACS). Astrocytes were separated from 6-12 week old mouse brain by Anti-ACSA-2 (astrocyte cell surface antigen-2) MicroBeads. The separation yielded 4.1x10E5 ± 0.7x10E5 astrocytes with a viability of 71.5 ± 8.8% and a purity of 95.2 ± 3.8%. In case of neurons, an untouched isolation strategy was applied by magnetic labeling and depletion of non-target cells using a mixture of non-neuronal cell marker. 2.0x10E5 ± 0.25x10E5 neurons with a viability rate of 72.0 ± 7.1% and a purity of 92.2 ± 1.1% were acquired per adult mouse brain. Likewise, 1.2x10E5 ± 0.25x10E5 oligodendrocytes with a viability of 76.7 ± 10.3% and a purity of 90.0 ± 6.7% were obtained using Anti-O4 MicroBeads.

Cultivation conditions were carefully optimized for all cell types. Immunocytochemical staining experiments showed highly pure cultures for astrocytes (Fig.1A), oligodendrocytes (Fig.1B), and neurons (Fig.1C) with a low percentage of contaminating cells after 7 days *in vitro*.

Taken together, we developed a standardized process that allows gentle automated dissociation of adult rodent brain tissue and convenient isolation of viable astrocytes, oligodendrocytes, and neurons using a magnetic cell sorting approach. Isolated cells can be cultivated and applied to study the function of individual adult neural cells at the morphological and molecular level.

Image



T02-09A

Self-renewal capacity of reactive astrocytes *in vivo*

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Stem cells are characterized by long-term self-renewal. Cells akin to astrocytes act as adult neural stem cells (NSCs) in specific niches of the adult mammalian brain. While these cells self-renew only for a limited number of cell divisions *in vivo* (Calzolari *et al*., 2015; Encinas *et al*., 2011), they have the potential to self-renew for a long time in the neurosphere assay *in vitro*. Astrocytes in the healthy brain parenchyma do not proliferate, but invasive injury triggers both proliferation and activation of NSC potential (neurosphere formation) in a subset of parenchymal astrocytes in the cerebral cortex gray matter (GM) (Buffo *et al*., 2008; Sirko *et al*., 2013; Götz & Sirko, 2013; Dimou & Götz, 2014). Again, reactive astrocytes have been observed to undergo maximally a single cell division *in vivo* (Bardehle *et al*., 2013), but *in vitro* in the neurosphere assay a small subset of reactive astrocytes can self-renew for much longer. The aim of this study was to assess whether and to which extent a larger number of astrocytes *in vivo* could be stimulated to proliferate and, in particular, whether the same astrocytes may proliferate several times. To address this question, we analyzed the acquisition of cycling activity of reactive astrocytes through their response to repetitive pathological events in the adult cerebral GM. For this purpose we used a dual-labeling method to follow cycling cells during S-phase *in vivo* by combining two thymidine analogues, 5-bromo-2'-deoxyuridine (BrdU) and 5-ethynyl-2'-deoxyuridine (EdU) at different time points after stab wound injury in the somatosensory GM. Quantitative analysis of *in vivo*-labeled cycling cells showed that repetitive pathological stimuli induced marked changes in the proliferative behavior of reactive astrocytes and other glial cells. Moreover, the changes in astrocytic proliferation observed *in vivo* correlated with the stem cell potential of these cells *in vitro*. These results bring new insight into the injury-induced plasticity of reactive astrocytes *in vivo* and how this potential might be unleashed in a pathological environment.

T02-10A

Exogenous FGF-1 hault differentiation of NKX2.2+pre- OPC to NG2+ OPC in a rat spinal cord transection model

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FGF-1 is a member of the FGF1,2 sub-group of FGF family. It was reported to inhibit OPC differentiation in vitro. FGF-1 activate all 4 types of FGF receptors, and is neuroprotective after spinal injury, and neurons from various CNS regions. An surgical procedure on the spinal transected rats

utilized the FGF-1 gained partial functional recovery (Cheng, Cao et al. 1996). Although pro-neuroregenerative, the effect of FGF-1 on oligodendrocytes in the transected spinal cord is not clear. Another similar member, FGF-2, regulate the generation of oligodendrocyte progenitor cells (OPC) from the embryonic tissue. FGF-2 is a mitogen for OPC, and inhibit the differentiation of oligodendrocyte lineage cells (OLC) to become mature oligodendrocytes. In this project we propose to study the effect of FGF-1 on cells of oligodendrocyte lineage in the transected spinal cord, to better understand the mechanism of FGF-1 on neuron and in glial cells in the injured spinal cord. The SD rats were transected at the T8 spinal cord level and the 0.5cm segment of spinal cord removed (the T group). In some rats the transection sites were treated with FGF-1 mixed in the fibrin glue (the F group). In some rats of the F group FGF-R1 blocker PD173074 were mixed with the FGF-1 in the fibrin glue and applied at the transection site (F+blocker group). Result: the back-degenerative site of the dorsal funiculas undergo generation of NG2+ Ox42- oligodendrocyte progenitor cells (OPC) within 4 days in rats of the T group. The number of NG2+ Ox42- OPC In the F group were reduced compared to the T groups. In rats of the F+ blocker group the NG2+ OX42- OPC number was similar to the rats of the T groups, and significantly increased compared to the F group. This data suggested that blocking of FGF receptor promote the maturation of pre-OPC to become NGs+ OPC.

T02-11A

DMT1 is expressed and required for adequate maturation in oligodendrocytes

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Introduction: Myelination takes places during early postnatal life in rodents. This process requiresoligodendrocyte precursor cells (**OPCs**) to experiment phenotypic changes in order to accomplish a maturation program that finally yields mature myelinating oligodendrocytes. Divalent Metal Transporter 1 (**DMT1**) is a multi-metal transporter whose primary role in iron transport (1) has been previously described inCNSdevelopment, but whose participationin oligodendrocyte maturation and myelinationremains unknown (2).

Results: The present work shows that only DMT1 isoform B is present in OPCs and *in vitro* experiments demonstrate that DMT1 is upregulated during OPC development.Western blot and RT-PCR performed on primary oligodendrocyte cultures show low levels of DMT1 expression in OPCs but higher quantities in mature oligodendrocytes. Co-localization of DMT1 with oligodendrocytedifferentiation markers reveal that MBP-, CC1-, O1- and O4-positive cells express high levels of DMT1, while PDGFrα-positive cells show low expression ofthis metal transporter.

Similar conclusions can be drawn from*in vivo* experiments, as DMT1contents increase in the corpus callosum, cortex and cerebellum during postnatal myelination in the mouse brain. Furthermore, DMT1 is highly expressed by myelinating oligodendrocytes during the development of the corpus callosum and cortex inEGFP-expressing mice under the control of PLP promoter. Interestingly, the spinal cord shows decreasing levels of DMT1 content during the time points analyzed.

Toward further elucidating the role of DMT1 in oligodendrocyte maturation, siRNA-mediated silencing of DMT1experiments carried out in primary cultures of OPCs prove that reduced levels of DMT1 significantly delay OPC development. DMT1 knockdown in turn induces a decrease in the proportion of myelin-protein-expressing oligodendrocytes and an increase in cells retaining immature oligodendrocyte markers.

Conclusions: Our data suggest that DMT1 is an important multi-metal transporter for proper oligodendrocyte maturation. We have found that DMT1 is upregulated during the development of OPCs and highly expressed by myelinating cells in the postnatal mouse brain. More importantly, the absence of DMT1 blocks OPC maturation *in vitro*.

T02-12A

NG2 and S100B co-localization in the developing mouse hippocampus

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Besides astrocytes and oligodendrocytes, NG2 proteoglycan expressing cells represent a third subtype of macroglial cell in the brain. Originally described as oligodendrocyte precursor cells, they feature several characteristics not expected from mere progenitor cells, including synaptic connections with neurons. Recent studies indicate that different subpopulations of NG2 glia exist in different regions and depending on the developmental stage. For example, subsets of NG2 glial cells express S100B, a typical marker for mature astrocytes (Karram et al., Genesis 2008, 46:743-572).

Here, we examined the expression profile and the co-expression of NG2 and S100B in the hippocampus and *corpus callosum* of juvenile and adult Balb/c mice (P10-12, P25-35, P80-90) using immunohistochemistry. To verify the specificity of the antibody for NG2, NG2-/- animals (Karram et al. 2008) were immunohistochemically labelled for NG2 versus their heterozygous littermates. Labelling for NG2 derived from NG2-/- mice resulted in staining pattern similar to what was found in the negative control labels. In the NG2-EYFP heterozygous littermates, in contrast, high intensity signal levels with co-localisation degree of 97% was obtained for the NG2 antibody.

In both the hippocampus proper and the *corpus callosum,* the number and density of NG2 cells was highest at P10-12, and was decreased by about 50% at both P25-35 and P80-90. At the same time, co-expression of NG2 and S100B increased from 7% at P10-12 to 27% in P25-35 animals in the *stratum radiatum* and *stratum oriens*. In the dentate gyrus and *corpus callosum*, in contrast, a constant low number of NG2/S100B cells (stratum radiatum of the more mature groups, 2-3% of NG2/S100B cells also expressed Ki-67.

Taken together, the results show a substantial regional and developmental heterogeneity in S100B expression by NG2 cells. Furthermore, they suggest that -in contrast to S100B expressing astrocytes- NG2/S100B cells still have proliferative potential.

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T02-13A

Role of microRNAs miR-124 and miR-137 in directing neuronal reprogramming of astrocytes

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Utilization of endogenous brain cells that can trans-differentiate to neurons remains a challenge with important clinical applications such as treatment of brain injury and neurodegenerative diseases. In that context, recent studies have demonstrated that brain astrocytes can be reprogrammed to neural stem cells and neurons mainly by overexpression of neural specific transcription factors, such as Mash1 and Neurogenin-2 (Ngn2). In this study we examined the potential role of neural specific microRNAs in the direct reprogramming of astrocytes to neurons. MicroRNAs (miRNAs) have emerged as critical post-transcriptional modulators of gene expression and are believed to be implicated in the regulation of virtually all biological processes, including neuronal development, by targeting large numbers of genes simultaneously. Thus they appear as attractive candidates for the regulation of both neural stem cell lineage progression and direct reprogramming between cell types of the same lineage through fine tuning of neurogenic factors expression levels. For this purpose we investigated the role of two brain-enriched miRNAs, miR-137 and miR-124, in the direct reprogramming of primary mouse astrocytes to neurons. We found that miR-137 overexpression led to the conversion of astrocytes to a radial glial phenotype, whereas overexpression of miR-124 alone led

to the formation of βIII-tubulin- and MAP2-positive neurons. Part of these neurons were also expressing GABA, but were negative for other subtype-specific markers, such as glutamate and tyrosine hydroxylase (TH). However, when we over-expressed the proneural bHLH transcription factor Ngn2 along with miR-124, we detected TH-positive neurons, indicating that miR-124 is sufficient to establish a general neuronal phenotype in induced neurons derived from astrocytes, but additional overexpression of neural specific transcription factors is required to confer subtype specificity. Recently, we have constructed a miR-124-GFP lentiviral vector to elucidate the in vivo effect of miR-124 alone or in combination with Ngn2 in reprogramming activated cortical astrocytes towards the neuronal lineage, by stereotaxically injecting the appropriate viral vectors in the astrogliotic area of a mouse traumatic brain injury model.

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T02-14A

Oligodendrocyte maturation through gestational iron deprivation

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Iron deficiency (ID) is, according to the WHO, the most common nutritional deficiency in the world and the second one in economic impact. This deficiency affects oligodendrocyte (OL) maturation, causing hypomyelination which continues in adulthood even after normal iron diet reinstatement. In this context, our fundamental aim is to elucidate the role of iron in the myelination process and iron acquisition mechanisms by OL and/or their progenitors (OPC). We have previously demonstrated that gestational ID produces alterations in myelin composition, abnormal migration and maturation of OPC and altered myelin structure. To further describe the whole population of OL, we have now focused on four aspects: a) the expression pattern of markers for different stages of OL lineage along ontogenetic myelination, b) OL morphological architecture, which reflects OL maturation, c) the timing of dysmyelination in different brain areas and d) OL interaction with different cell types within normal CNS cytoarchitecture, such as nodal and paranodal distribution and structure and GAP junction integrity and pattern. We use an eGFP::CNPase transgenic mouse experimental model, whose green-fluorescent OL-lineage-committed cells (CNPase-positive cells) allow the visualization of the entire myelin structure, as well as the analysis of single OL morphology and population features. Pregnant mice were fed an iron-deficient diet (4mg/g/kg) as from gestational day 5 until pup weaning (post-natal day 21, PND21). Plates within the anterior-posterior axis were evaluated at PND15 and 30, and CNPase-positive cell distribution was quantified in optic nerve, cortex, striatum and corpus callosum. Control animals evidenced an increase in OL complexity both during ontogenetic development and along the anterior-posterior axis; in particular, OL population exhibited the highest mature percentage at posterior position. In turn, ID animals exhibited fewer eGFP-CNPase-positive cells, with prevalence of immature OL, as tested by specific markers and confirmed by a decrease in MBP expression. These results correlate with behavioral alterations observed both during development and young adulthood. We conclude that low-iron availability does not affect cell lineage specification but expands an arrested OPC population, which is responsible for hypomyelination.

T02-15A

Ependymal cilia polarization and IIIG9 expression is a synchronous process

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Introduction: IIIG9 is a protein of unknown function; its localization is specific to the cilia of the trachea epithelium, fallopian tubes and testicles. Thus, IIIG9 may play a role in the polarization of ciliated cells. In this study, we produced specific antibodies against IIIG9 and analyzed its expression during *in situ* and *in vitro* ciliated ependymal cell polarization in rat brain development.**Material and Methods:** Anti-IIIG9 antibodies were characterized by optical, confocal spectral microscopy and SIM super resolution microscopy. Acetylated-tubulin (cilia marker) and vimentin (ependymal marker) were

also used in embryonic (E17), postnatal (P1-P20) and adult samples. IIIG9 mRNA expression was analyzed by QRT-PCR in samples obtained by LMD of ependymal cells and different subpopulations of hypothalamic tanycytes. Finally, we established a primary cell culture of isolated ependymocytes to determine IIIG9 expression during the ependymal phenotype (vimentin +; 4 DIV).**Results:** In adult brain, IIIG9 mRNA is only expressed in ependymocytes but not in tanycytes. During brain development, IIIG9 is absent in E17-P1 stages, but it is detected in P10-P20. This protein colocalized in these stages synchronously with the acetylated tubulin and vimentin. Additionally, using primary culture cells and brain tissue samples, we demonstrate that IIIG9 is principally localized in the cilia of the ependymocyte cells following a dotted pattern (SIM super-resolution microscopy).**Conclusion:** IIIG9 expression and the ependymal cilia polarization is a synchronous process that occurs early during postnatal life in the rat brain. Funded by FONDECYT (Initation into Research Competition) 11140405, DIUC 214.031.110-1.0 and CMA BIOBIO, PIA-ECM-12.

T02-16A

Postnatal hyperoxia affects OPC and GCP proliferation

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Preterm birth is often causing injury of the white matter and impairment of neurological development in former preterm infants. Due to recent insights, brain injury after premature birth often includes reduced growth of the cerebellum with injury found in neural cell layers and in white matter regions. Generally, perinatal brain injury may be caused by ischemia, infection/inflammation, or hyperoxia. However, reasons for longterm impairment of the cerebellum are undefined. In this study, we analyzed the effects of postnatal hyperoxia on oligodendrocyte precursor cells (OPC) in the white matter and on granule cell precursors (GCP) in the external granule cell layer (EGL) of the cerebellum.

We used a rodent hyperoxia model exposing newborn rats to 80% O_2 for 24h from P6-P7. Nitrotyrosine Western blot analysis of whole cerebellar hemisphere was performed to detect oxidative stress. Cerebellar gene expression of the proliferation marker Cdk2, the mitogen shh and the growth factor Pdgfa was analyzed by Real Time qPCR. Immunohistochemistry with antibodies against NG2 and Ki67 as well as against Pax6 and PCNA was performed to analyze the proliferation of OPCs (NG2+) and GCPs (Pax6+) in the cerebellum. MRI was used to measure cerebellar volume in young adult rats at P30.

As a result, hyperoxic rat pups had significantly higher levels of nitrotyrosine at P7. Gene expression of proliferation marker Cdk2 in the cerebellum was downregulated by hyperoxia at P7. Both shh, which is known to drive GCP proliferation, and Pdgfa being responsible for OPC proliferation, were significantly reduced by hyperoxia at P7. Numbers of proliferating OPCs co-labeled with NG2 and Ki67 were decreased after hyperoxia. Also, the ratio of Pax6+ GCPs being positive for proliferation marker PCNA was reduced in the EGL. After recovery in room following the hyperoxia exposure, juvenile rats at P30 had reduced cerebellar volume as compared to control litters always kept in room air.

In our study in rats, postnatal hyperoxia caused oxidative stress in the neonatal cerebellum and perturbed proliferation of OPCs and GCPs. Based on these results, we postulate that maldevelopment of the cerebellum found in preterm infants can be caused by postnatal oxygen toxicity.

T02-17A

Umbilical cord blood stem cells-derived microglia

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Recent reports indicate that microglia originate from immature yolk sac progenitors. After birth, microglia maintain their numbers under normal conditions by self-renewal without the recruitment of

monocyte-derived microglial precursors. Only disruption of the blood-brain barrier can allow peripheral cells to invade the brain parenchyma and maintain as microglia-like cells.

In this study, we report an autopsy case in which cells derived from umbilical cord blood could differentiate into ramified microglia in the host brain parenchyma without brain irradiation. Although the blood-brain barrier and glia limitans prevented the invasion of these donor-derived cells, most of the invading donor-derived ramified cells maintained in the cortical zone. This result suggests that the invasion of donor-derived cells occur through the pial membrane.

T02-18A

SNX27 regulation of GPR17 recycling is important for the correct differentiation of oligodendrocytes

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During the development of oligodendrocytes, G protein-coupled receptor 17 (GPR17) acts as an intrinsic timer that regulates the transition of immature oligodendrocyte precursor cells (OPCs) to pre-myelinating oligodendrocytes but, although it is required for the initial differentiation of OPCs, it has to be down-regulated to allow final myelination. We have previously studied GPR17 endocytic trafficking in oligodendroglial cells and found that, upon agonist-mediated internalization in early endosomes, sorting between recycling to the plasma membrane or degradation in lysosomes determines whether receptor signalling is sustained or switched off. To characterise the mechanisms controlling GPR17 intracellular sorting further, we investigated the role of the PDZ binding motif at the C-terminal of the receptor, and found that disrupting this motif by means of site-directed mutagenesis increases the intracellular retention of GPR17 in endocytic vesicles, thus indicating that the C-terminal domain is involved in receptor trafficking. On the basis of this finding, we looked for PDZ proteins that interact with and may regulate the trafficking of GPR17. Pull down experiments showed that SNX27 (a major regulator of receptor sorting to the recycling pathway) directly binds the GPR17 C-terminal and, in line with this, GPR17 recycling was impaired in SNX27 knock-down cells (upon stimulation, 20% more receptors were retained in the SNX27 knock-down cells than in cells transfected with scrambled RNA). Notably, the loss of SNX27 significantly decreased the levels of GPR17 (Fig C and F) and, as the levels of GPR17 mRNA were not decreased, we concluded that SNX27 knock-down accelerates GPR17 degradation, as previously reported for other receptors. These observations led us to investigate whether the early down-regulation of GPR17 signalling plays a role in oligodendrocyte maturation, and found that the reduction in GPR17 levels correlates with an increase in the expression of the MAG (Fig B and E) and MBP myelin proteins. These findings demonstrate for the first time that SNX27 is involved in GPR17-mediated signalling and, consequently, oligodendrocyte differentiation. As SNX27 is down-regulated in patients with Down syndrome, these results suggest a possible link between the loss of SNX27 and the myelination defects in the brains of such patients

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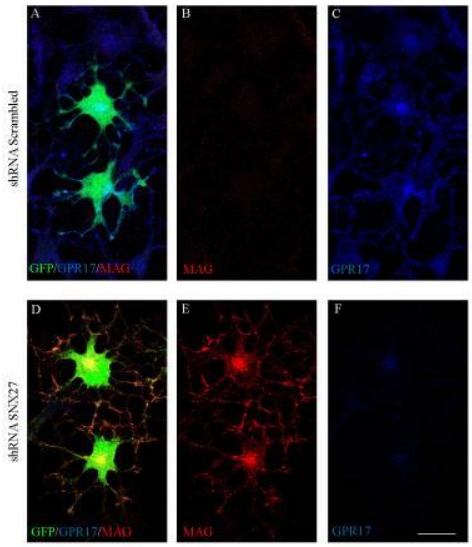
A.F.U. and V.M. contributed equally to this study.

Image



T02-19A

Role of Ascl1 in NG2 cells in the embryonic and adult spinal cord

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NG2 cells (also known as oligodendrocyte precursor cells) are one of the major glial cell populations in the CNS, are highly proliferative, and are widely distributed into both gray matter (GM) and white matter (WM). Throughout life, NG2 cells can readily differentiate into oligodendrocytes to myelinate axon fibers, or they can remain as NG2 cells and directly interact with neurons. Recently, it has been shown that deletion of tumor suppressor genes specifically within NG2 cells was sufficient to produce brain tumors in mouse models, indicating that NG2 cells may serve as a potential cell of origin for gliomas. At present, however, understanding of the molecular mechanisms that regulate NG2 cell proliferation and differentiation in the CNS remains incomplete. Interestingly, Ascl1, a basic-helix-loop-helix (bHLH) transcription factor that is essential for neural development, is highly expressed in NG2 cells but not in mature oligodendrocytes. Although previous studies have shown that the loss of *Ascl1* affects the initial specification and differentiation of NG2 cells, the specific function of Ascl1 in NG2 cells during embryonic and postnatal development remains unknown.

In this study, we investigated the direct requirement of *Ascl1* in the development of NG2 lineage cells in the GM and WM of the embryonic and adult spinal cord. In particular, we conditionally deleted *Ascl1* specifically within NG2 cells (Ascl1-CKO) at E14.5 or at P30 using an *NG2-CreERT2* mouse strain in which the tdTomato (tdTom) fluorescence reporter was also incorporated to allow direct visualization of the development of NG2-labeled (tdTom+) cells at 7, 28, and 56 days post-tamoxifen injection (dpi). We found that Ascl1-CKO at E14.5 initially decreased the number of NG2-lineage tdTom+ cells in both the GM and WM at 7 dpi, but by 28 dpi, the WM NG2-lineage tdTom+ cells had increased in number to reach a level similar to that of the control littermates. In contrast, Ascl1-CKO at P30 resulted in a consistent reduction in the number of NG2-lineage tdTom+ cells in both the GM and WM at 28 and 56 dpi. Quantification of the percentage of NG2-lineage tdTom+ cells that had proliferated (BrdU+, Ki67+) or differentiated (CC1+) into oligodendrocytes revealed that Ascl1-CKO at E14.5 decreased the proliferation of GM NG2 tdTom+ cells but extended the proliferation of WM NG2 tdTom+ cells, resulting in an increase in the proportion of WM NG2 tdTom+ cells that were proliferating but also expressing the mature oligodendrocyte marker CC1. By contrast, Ascl1-CKO at P30 decreased the proliferation and accelerated the differentiation of NG2 tdTom+ cells in both GM and WM compared to controls. Taken together, these findings indicate a crucial role for Ascl1 in ensuring the proper number and development of NG2-lineage cells in the GM and WM of the spinal cord.

T02-20A

Dynamic regulation of Olig2 expression in oligodendrocyte differentiation

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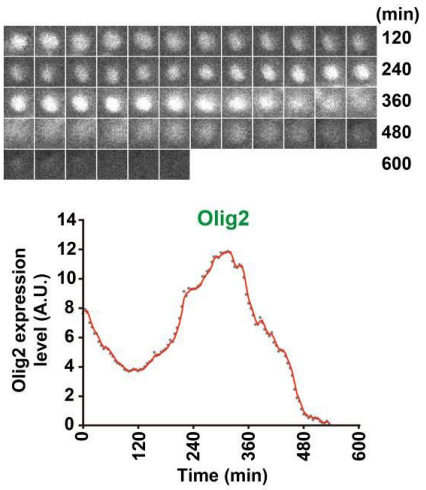
The majority of oligodendrocytes are generated from proliferative and migratory oligodendrocyte precursor cells (OPCs) in the embryonic and postnatal brain. However, OPCs continue to proliferate and give rise to new-myelinating oligodendrocytes throughout adulthood. Previous studies showed that OPCs express NG2 proteoglycan, and these cells generate NG2 cells themselves or oligodendrocytes in the adult brain, whereas in the embryonic and early postnatal brain, they can also generate astrocytes.

The bHLH transcription factors Ascl1, Hes1, and Olig2 regulate fate choice of neurons, astrocytes, and oligodendrocytes, respectively. These factors are coexpressed by neural stem (NS) cells. Recently, we found by time-lapse imaging that these factors are expressed in an oscillatory manner by NS cells. Herein, we focused on Olig2 expression dynamics. We found that Olig2 protein expression oscillated in cultured self-renewing NS cells. Olig2 protein oscillated more slowly (average period of 6-8 hours) than Hes1 and Ascl1 protein oscillation. When NS cells were induced to oligodendrocyte, Olig2 protein expression still oscillated but at high trough levels during OPC state. But a few days after oligodendrocyte induction, Olig2 expression was suddenly down-regulated, and the expression of mature oligodendrocyte marker CNPase was followed. We generated cultured NS cells from *Olig2* KO mice, and these cells showed a reduced ability to generate oligodendrocytes. We are planning to adapt newly developed optogenetics (Photo-acivatable Gal4/UAS system) to control Olig2 expression dynamics in *Olig2*-null NS cells, and examine the effects of oscillatory and sustained Olig2 expression on OPC proliferation and oligodendrocyte differentiation.

Image



T02-21A

Ex-vivo analysis of astrocyte subpopulations

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Astrocytes are known to perform multiple functions in the central nervous system, including regulation of inflammatory reactions, neuronal support, fluid and ion homeostasis and glial transmission. The wide-ranging variety of complex astrocyte functions raises the question whether multiple subtypes of astrocytes exist. Indeed increasing evidence supports heterogeneity of the astrocyte population. Nevertheless, the heterogeneity of astrocytes has not been comprehensively explored yet. In order to get more insights into different astrocyte subtypes, we have established a fluorescence-activated cell sorting (FACS) based protocol for the isolation of pure populations of astrocytes from adult mice. Several subpopulations of GFAP-, Glast- and GLT1-positive astrocytes could be isolated from different brain regions and the spinal cord. With the aim of studying the differences between these astrocyte subpopulations, these ex-vivo astrocyte populations will be further analysed by high-throughput RNA sequencing, complemented by quantitative RT-PCR, immunohistochemistry and Western blotting.
Characterisation of these astrocyte populations will lead to a better insight into the heterogeneity of astrocytes. Furthermore, our FACS-based isolation technique can be used to isolate astrocyte subpopulations from a multitude of mouse models, leading to a better understanding of the phenotypes of astrocytes, not only during health, but also during disease.

T02-22A

OPC heterogeneity in the optic nerve

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In the embryonic murine central nervous system (CNS) it has been shown that OPCs are generated in different waves along a spatiotemporal gradient; with ventral OPCs emerging before dorsal OPCs. These developmentally distinct OPC populations show functional differences in remyelination of the adult CNS, and therefore serve as an excellent model system to identify general mechanisms influencing remyelination. Using a *Sox10*-driven reporter mouse model, which allows the ventrally- and dorsally-derived OPC populations to be distinguished based on their differential expression of fluorescent proteins, we found that ventrally- and dorsally-derived OPCs are present in the optic nerve. While the ventrally-derived population dominates the optic nerve, the percentage contribution of ventrally-and dorsally-derived OPCs differs along the rostro-caudal axis of the optic nerve. Furthermore, the number of both ventrally- and dorsally-derived OPCs decreases significantly with ageing. To evaluate if ventrally-derived OPCs can functionally compensate for dorsally-derived OPCs, myelination will be assessed in a mouse line in which the dorsally-derived population was ablated using cell-specific diphtheria toxin expression.

T02-01B

Profiling of the different genes regulated during astrocyte differentiation

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Introduction and purpose: Astrocytes, the most abundant glial cell population, are now emerging as key players in many aspects of brain function, homeostasis and neurological disorders. Astrocytes constitute a heterogeneous group of cells and differ in their function, morphology, physiological properties and response to injury and disease.
Some studies showed the high plasticity of astrocytes. In fact, astrocytes can be reprogrammed in vitro to generate neurons (Heinrich, 2010). They can undergo dedifferentiation upon injury and inflammation and may acquire the potentiality of neural progenitors (Yang, 2012; Buffo, 2010). However, the investigations concerning the heterogeneity and plasticity of astrocytes are limited due to the lack of mature astrocytic markers. New markers of astrocyte maturation will help to study astrocytic diversity, function and theirs implication in neurological disorders.
In the present study, we want to deliver a more complete knowledge on the transcriptomic and protein signatures of the astrocyte development and so investigate the different markers expressed by mature astrocytes.

Methods: Primary cultures of neurospheres (NSP) were obtained from prenatal mouse brains as described previously (Grandbarbe, 2003) and were differentiated into astrocytes with 10% fetal bovin serum. At different times of differentiation, a transcriptomic profile was established by microarrays and RT-PCR in order to investigate the different markers expressed during astrocyte differentiation. Protein analysis were done by immunohistochemistry.
These results were also confronted to the microarrays analysis of pure primary astrocytes. Cultures of primary mouse astrocytes were prepared from brains of postnatal C57BL/6 mouse (P0-P2). When glia cells reached confluence, a magnetic separation (MACS technology) was carried out and primary astrocytes were negatively sorted using an anti-CD11b antibody.

Results and conclusion: Our preliminary results suggest that astrocytic differentiation occurs in several steps associated with specific expression profiles, markers and signaling pathways. Moreover, a mature astrocytic profile can be established following the comparison of results obtained with NSP derived astrocytes and primary astrocytes. Similar results were found in both models. In fact, genes upregulated during astrocyte differentiation and characteristic of the astrocyte maturation step were also strongly expressed by primary astrocytes. These new signatures of astrocyte maturation will help to address astrocyte diversity and plasticity in the adult brain.

T02-02B

Phenotypic heterogeneity of dividing oligodendrocyte progenitor cells and of their progeny: characterization and modulation by aging and extrinsic factors

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Oligodendrocyte progenitor cells (OPCs) persist in the adult Central Nervous System and guarantee oligodendrocyte turnover throughout life. It remains obscure how OPCs avoid exhaustion during adulthood and whether they self-maintain by undergoing asymmetric divisions generating a mixed progeny either keeping a progenitor phenotype or proceeding to differentiation. To address this issue, we examined OPCs during mitosis and sister cells after cell birth. In these cells we assessed the distribution of transcription factors (TFs) related either to cell stemness or to progression toward oligodendrogenesis, and of stage-specific markers, and investigated their correlation with distinct short-term fates. We found that distinct TFs are expressed in segregated subsets of mother OPCs in both the adult and juvenile cerebral cortex. Moreover, we showed that a fraction of dividing OPCs gives rise to sister cells with diverse immunophenotypic profiles and short-term behaviours. This latter heterogeneity appears as cells exit cytokinesis, but does not derive from the asymmetric segregation of molecules such as NG2 or PDGFRa expressed in the mother cell. Rather, rapid downregulation of OPC markers and upregulation of molecules associated with lineage progression contributes to generate early sister OPC asymmetry. Analyses during aging and upon exposure to physiological (i.e. increased motor activity) and pathological (i.e. trauma or demyelination) stimuli showed that both intrinsic and environmental factors contribute to determine the fraction of symmetric vs. asymmetric OPC pairs and the phenotype of the OPC progeny as soon as cells exit mitosis.

Yet, it remains to be proved whether subsets of OPCs expressing distinct TFs are committed to produce different progenies (i.e. symmetric vs. asymmetric, or differentiating vs. maintaining a progenitor phenotype), or, alternatively, whether distinct features in mother cells just reflects a transient and stochastic state in response to environmental cues. Ongoing experiments are addressing these issues by in vivo fate-mapping approaches and alterations of environmental regulatory candidates.

T02-03B

Microglial cells during embryonic development of the mouse brain - mature team players or young bench sitters?

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Microglial cells are the innate immune cells of the central nervous system. They appear early in the embryonic brain and spinal cord and are important for their normal development. Time-lapse analysis showed that embryonic microglia are highly dynamic cells. Suggesting that these cells are already very active during fetal development. In addition, we have shown that these cells accumulate in the choroid plexus primordium, where they are in the proximity of dying cells. Their active behavior and morphological characteristics suggest they are in an alerted/activated state during embryonic development. Besides immunohistochemical markers is the presence of different K+ channels on microglia also an indication of their activation stage. However most studies have been conducted on postnatal and adult microglial cells. Therefore we aimed at determining the electrophysiological activation phenotype of these embryonic microglia.

At the age of E13.5, cortical microglia display few to no inward rectifying K+ current and this independent of their location in the embryonic cerebral cortex and their cell morphology. These cells also express functional P2X receptors, which based on the profile of the response are most probably P2X7 receptors. In the choroid plexus, where they are in close proximity of dying cells, the microglia expressed an inward rectifying K+ current suggesting they are in an “alerted/activated” state. In addition, cell death was still observed in the choroid plexus after elimination of microglial cells, indicating they are not needed to trigger apoptosis of choroid plexus cells.

T02-04B

Calmodulin inhibition affects proliferation and cell viability in unchallenged and LPS-challenged pure microglial cultures

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Several aspects of cell proliferation and cell viability were tested after treatments with different calmodulin inhibitors (calmidazolium (CALMID) and trifuoperazine (TFP)) in pure microglial cell cultures. Mixed primary cortical cell cultures were established from E18 wild-type rat embryos and maintained routinely in cultures for up to 28 days (DIV1-DIV28). Pure microglial cell cultures were subcloned from mixed primary cultures (DIV7). The medium was changed on the first day after seeding (subDIV1). For the immunological detection of different microglial phenotypes developed in vitro, an antibody against Iba1, an intracellular actin- and Ca^{2+}-binding protein expressed in macrophages and microglia. Immunocytochemistry routinely performed on the pure microglial cultures 4 days after seeding (subDIV4) consistently detected a >99% incidence of Iba1-immunopositive microglia for the Hoechst 33258 dye-labeled cell nuclei. An anti-CaM monoclonal antibody was used to detect both Ca^{2+}-bound and Ca^{2+}-free forms of the antigen. Phalloidin, a bicyclic heptapeptide that binds only to filamentous actin, was used to visualize F-actin bundles in the microglia. The anti-Ki67 antibody was used to detect proliferating cells. Ki67 is a nuclear protein expressed in all active phases of the cell cycle from the late G1 phase through the end of the M phase but is absent in non-proliferating and early G1 phase cells. The anti-glyceraldehyde 3-phosphate dehydrogenase (GAPDH) antibody was used as an internal control in Western blot experiments. When CaM inhibitors were tested on cell proliferation and cell viability, CALMID and TFP, either alone or in combination with LPS, had different effects. Proliferation index (PI) was measured as a function of Ki67-immunopositivity of the cells in 1,000 analyzed microglia in culture. Unstimulated microglia (subDIV4) had an average PI value of 2.5% indicating the presence of an actively proliferating subdivision of microglial cells. LPS challenge inhibited cell proliferation (PI = 0.41), albeit without reaching significance. While CALMID was ineffective, TFP10 significantly decreased proliferation both in unchallenged and LPS-challenged cells with PI values of 0.21% and 0.12%, respectively. Cell viability studies showed that in contrast with the ineffectivity of CALMID50 on cell survival in unchallenged and in LPS-challenged microglial populations, TFP10 was highly effective in these cultures as it significantly decreased cell viability to 62.47% and 71.28% of the control values, respectively.

T02-05B

Rapid and efficient generation of human oligodendrocytes from induced pluripotent stem cells

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The ability to generate human oligodendrocytes from pluripotent stem cells *in vitro* offers a promising approach to study the underlying mechanisms of oligodendrocyte specification, maturation and myelination. In addition, the use of patient-specific induced pluripotent stem cells (iPSCs) could provide new insights into human demyelinating diseases as multiple sclerosis and leukodystrophies. However, oligodendrocyte differentiation protocols from human iPSCs are still inefficient and very time-consuming with 75 to 150 days in culture limiting the applicability of this technique.

In this study, we generated human neural progenitor cells (NPCs) from iPSCs and sought to identify transcription factors (TFs) governing oligodendroglial specification and differentiation. Our screen revealed a combination of three TFs inducing a rapid and efficient oligodendroglial cell fate in human NPCs. By overexpressing these TFs in NPCs we were able to rapidly induce human oligodendrocytes (iOLs) with an efficiency of 40-50% O4-positive cells at day 28. We further showed that these cells express several oligodendroglial markers and differentiate into mature MBP-expressing oligodendrocytes. Moreover, *in vitro* myelination assays highlighted the capability of iOLs to myelinate human iPSC-derived neurons.

T02-06B

Identifying the ligand of adhesion-GPCR GPR56 in oligodendrocyte development and CNS myelination

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The family of adhesion G protein-coupled receptors (aGPCRs) facilitates cell-extracellular matrix (ECM) interactions. GPR56 is a member of the aGPCR family and its mutations are responsible for a human brain malformation known as bilateral frontoparietal polymicrogyria (BFPP). Individuals with BFPP present with both cortical malformation and central nervous system (CNS) hypomyelination. Here, we show that *Gpr56* knockout mice also present with CNS myelination defects. Transmission electron microscopy analysis revealed a significantly fewer myelinated axons in of the corpus callosum and optic nerves of *Gpr56* knockout mice, whereas the number of axons are comparable between heterozygous and knockout mice. Further mechanistic studies revealed (1) that the number of mature oligodendrocytes is significantly reduced in the corpus callosum *Gpr56* knockout mice; (2) GPR56 is expressed in oligodendrocyte precursor cells (OPCs) but turned off in mature myelinating oligodendrocytes (OLs); and (3) deleting *Gpr56* results in premature cell cycle exit of OPCs. In sum, our data suggests an autonomous role of GPR56 in OPC proliferation and CNS myelination.

Structurally, aGPCRs differ from other GPCRs by the presence of an extremely larger extracellular N-terminal region that is separated from the 7-transmembrane region by a GPCR autoproteolysis-inducing (GAIN) domain that in most aGPCRs facilitates an auto-catalytic process to produce an N- and C- terminal fragments during the maturation process. GPR56 has a long and poorly characterized N-terminal fragment, allowing for the possibility of multiple binding partners that mediate cell-ECM interactions. Indeed, we have shown that collagen III is the ligand of GPR56 in the developing cerebral cortex, whereas tissue transglutaminase (TG2) is the binding partner of GPR56 in melanoma cells. We have identified TG2 as a potential ligand of GPR56 in oligodendrocyte development and are currently studying the effects of TG2 on CNS myelination.

T02-07B

RXR-VDR signaling regulates oligodendrocyte precursor cell differentiation

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Myelin sheaths are lost from CNS axons in demyelinating diseases. Upon recruitment of adult oligodendrocyte progenitor cells (OPC) and their differentiation into myelinating oligodendrocytes new myelin sheaths are formed within areas of injury in a process known as remyelination. Remyelination is highly efficient in the early stages of chronic demyelinating diseases such as multiple sclerosis. Nevertheless, remyelination efficiency declines with disease progression and advancing age, leaving demyelinated axons unprotected and vulnerable to degeneration. Understanding the key signaling molecules orchestrating oligodendrocyte progenitor cell (OPC) differentiation and remyelination may translate to regenerative therapies for demyelinating diseases.

We have identified RXRγ as a key positive regulator of OPC differentiation during remyelination (Huang et al., 2011). RXRγ is a nuclear receptor that forms a heterodimer with other nuclear receptors to activate downstream signaling cascades. Here we show that VDR is highly expressed in OPCs and mainly in mature oligodendrocytes during remyelination and it is an RXRγ binding partner within oligodendrocyte lineage cells. We demonstrate that the block of VDR signaling in OPCs by a VDR antagonist or via siRNA inhibits OPC differentiation *in vitro* and myelination and remyelination in cerebellar slice cultures. Treatment of OPCs with vitamin D, a VDR agonist, increases OPC differentiation while decreasing OPC proliferation *in vitro*. Moreover, the VDR antagonist abrogates the pro-differentiative effect of 9-cis-retinoic acid, an RXR agonist, indicating a dominant role for VDR in the heterodimer.

Our data reveal that VDR-RXRγ heterodimer, regulated by ligands such as Vitamin D, acts as a switch to initiate OPC differentiation and promote remyelination, suggesting a potential role for VDR-RXRγ as a therapeutic target for demyelinating diseases.

T02-08B

Characterization of Tensin3 (*Tns3*) function in oligodendrogenesis and remyelination

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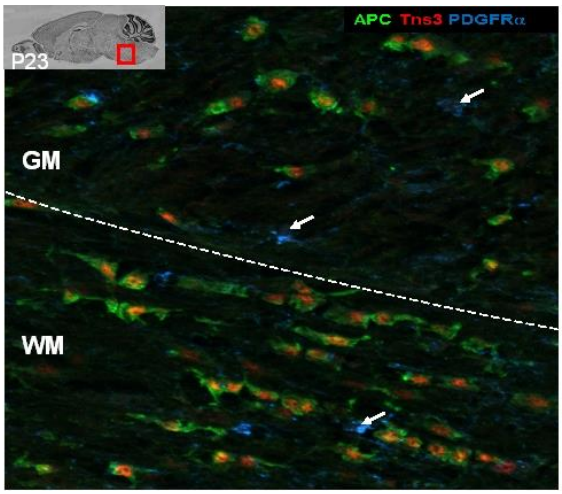
Multiple sclerosis (MS), one of the most prevalent neurological diseases, involves both immune and neural cells and it is characterized by the loss of myelin sheath enwrapping neuronal axons. Although several immune-focused treatment strategies have been developed they show only partial benefit, and efficient remyelinating therapies are still lacking. Endogenous oligodendrocyte precursor cells (OPCs) are present all over the adult brain of both MS patients and animal demyelinating models but their differentiation into new myelinating oligodendrocytes can be partially impaired. Therefore, there is a major need to develop treatments enhancing the process of myelin repair and thus, it is critical to understand the underlying mechanisms promoting (re)myelination. To this aim, we performed a screening for direct target genes of *Olig2 & Ascl1*, key transcription factors of oligodendrogenesis. Tensin 3 (Tns3) was found as a Ascl1&Olig2 direct target its mRNA expression being enriched in immature oligodendrocytes. Tns3 is a member of Tensin family which are focal adhesion proteins making connection between extracellular matrix and the actin cytoskeleton and it is believed that they play a role in cell proliferation, differentiation and migration.

To study its role in oligodendrogenesis, we first characterized its expression pattern in the mouse postnatal brain using an anti-Tns3 specific antibody. We found that Tns3 protein levels correlates with oligodendrocyte differentiation, being strong in early differentiating (immature) oligodendrocytes, weak in myelinating (mature) oligodendrocytes, and undetected in oligodendrocyte precursor cells (OPCs) during postnatal stages (from P7 to adult stages). Tns3 antibody specificity was confirmed using $Tns3^{LacZ}$ enhancer-trap mice (Su Hao Lu, UC Davis) where beta-galactosidase was similarly

expressed in maturing oligodendrocytes but not in OPCs. Moreover, Tns3 expression during remyelination (lysolecitin induced focal demyelination in the adult corpus callosum) correlated with new remyelinating oligodendrocytes in/around the lesion. We also found that other Tensin proteins (Tensin1 and Tensin2, also called Tenc1) were found in oligodendroglia. We are currently using the *Tns3*^{LacZ} mice to characterize the *Tns3* function in oligodendroglial cells in different regions of the brain (such as the Corpus Callosum and in the Fimbria). Gain-of-function experiments are also being conducted using *GFP-Tns3* or *GFP-Tns2* expressing plasmids in neural precursor/stem cultures.

Image



T02-09B

Cell fate of NG2 glia in the developing mouse spinal cord

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NG2 is a type I transmembrane glycoprotein and also known as chondroitin sulfate proteoglycan 4 (CSPG4). In the parenchyma of the central nervous system (CNS) NG2-expressing (NG2$^+$) cells have been identified as precursor cells of oligodendrocytes (OLs) in the developing white matter. However, several previous studies had suggested that in the spinal cord NG2 glia could also generate astrocytes. Since the respective transgenic mice had been generated by non-homologous recombination, harboring possible transgene insertion artifacts, we took advantage of our novel NG2-CreERT2 knock-in mice and the Rosa26-tdTomato reporter mice to investigate the differentiation potential of NG2 glia in the spinal cord during development (Huang et al., 2014).

(1) Cre activity was induced by tamoxifen administration at embryonic stages of E14.5, E17.5 and E19.5 and animals were analyzed postnatally. Up to postnatal day 21 (P21), in both grey and white matter of the spinal cord all the reporter-positive cells (except vessel-associated, recombined pericytes) displayed the oligodendrocyte lineage marker Sox10 immunoreactivity. We did not find any reporter-positive cells that expressed the astrocytic marker GFAP. Intriguingly, starting from P24, NeuN-positive/reporter-positive neurons started to appear in the grey matter of the spinal cord, though only very few (2-5 neurons per cross section of cervical spinal cord). (2) When tamoxifen was given to postnatal mice, most of the reporter-positive cells were either immunopositive for the oligodendrocyte precursor marker PDGFRα or the mature oligodendrocyte marker APC CC1, none of the reporter-positive cells expressed GFAP or demonstrated the bushy astrocyte morphology. However, still a few reporter-positive neurons were observed in the adult.

This study indicates that NG2 glia in the spinal cord are mainly restricted to the oligodendrocyte lineage and do not generate astrocytes at any developmental stage. The appearance of reporter-

positive neurons remains enigmatic: either a few NG2 glia acquire neurogenic potential or they deliver their cytosolic components to adjacent neurons as exosomes. Further experiments are required to investigate both scenarios.

Huang W, Zhao N, Bai X, Karram K, Trotter J, Goebbels S, Scheller A, Kirchhoff F (2014) Novel NG2-CreERT2 knock-in mice demonstrate heterogeneous differentiation potential of NG2 glia during development. **Glia** 62, 896-913.

T02-10B

Activity-dependent effects on oligodendrocyte precursors and mature oligodendrocytes in the adult sensorimotor cortex and corpus callosum

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Activity or social interactions including running, enriched environment (EE) or special reaching training (RT) are identified as high stimulators for grey and white matter plasticity in the adult brain. The present study focused on experience-dependent effects on oligodendrocyte precursor (OPCs) cells and mature oligodendrocytes in the sensorimotor cortex and corpus callosum of the adult rat brain. Therefore we used the following approach: one experimental subgroup was transferred to an enriched environment, a second group received daily reaching training of the dominant forelimb (reaching of 50 - 100 food pellets per day), whereas a third group remained in the standard cage. In order to analyse the proliferative response bromodeoxyuridine (BrdU) was applied at day 2 to 6 after beginning of the experiments. Animals were perfused at day 10 to assess ongoing proliferation of OPCs and at day 42 to analyse the survival of newly born oligodendrocytes in the sensorimotor cortex and corpus callosum. At day 10 proliferating NG2+ cells significantly decreased by EE housing compared to standard and RT, whereas proliferating NG2+GSTπ+ immature oligodendrocytes significantly increase after EE and RT. Furthermore 6 weeks of EE and RT significantly increase CNPase+ mature oligodendrocytes. Additionally RT significantly reduced the complexity of NG2+ cells between both time points measured by sholl analysis. The present results indicate that EE and RT promote the differentiation to oligodendrocytes in the sensorimotor cortex. These experience-dependent effects were not present on OPCs in the corpus callosum. We thereby provide *in vivo* evidence that proliferating OPCs in the sensorimotor cortex or in the corpus callosum differentially respond to external stimuli.

T02-11B

Mature astrocytes regain stem cell potential and give rise to neurons

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Mature astrocytes and neural stem cells share several phenotypic and functional features. From their resemblance, the idea was born that mature astrocytes might in return act as stem cells giving rise to neurons. Mature astrocytes have been shown to re-enter the cell cycle when exposed to defined stimuli. Astrocytes are also able to generate neurons, at least after genetic reprogramming. Moreover, astrocytic cells isolated from injured brains generated multipotent and self-renewing neurospheres. However, the sequential processes leading to the conversion of astrocytes to stem cells are highly complex and difficult to study at single cell resolution. Therefore a clean cell system would be desirable to show the direct generation of neurons from astrocyte-derived stem cells without genetic reprogramming.

We provide an *in vitro* model for the de-differentiation of astrocytes to neural stem cells by the exposure to defined growth factors. Astrocytes were generated from embryonic stem cell derived neural stem cells. They expressed markers of mature astrocytes like GFAP, Aqp4, and GLT-1 and reacted to inflammatory stimulation, a function which is typically found only in fully differentiated cells. By the addition of growth factors, they re-entered the cell cycle, upregulated markers of neural stem

cells like nestin and RC2, and lost their responsiveness to inflammatory cytokines. The de-differentiated cells were bipotent, giving rise to astrocytes and neurons.

Thus, we found a de-differentiation of astrocytes without genetic reprogramming in a clean *in vitro* system and showed that these de-differentiated cells are neurogenic stem cells. The homogeneity of the cell populations in this system strongly facilitates the search for mechanisms and signaling pathways involved in de-differentiation.

T02-12B

Interleukin-33 (IL-33) as a factor involved in the regulation of oligodendrocyte precursor cells biology

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Interleukin-33 (IL-33) is a protein which has been recently widely studied because of its an important role in maintaining organism homeostasis by dual activity - it may act as a traditional cytokine or as intracellular nuclear factor.

The aim of present study was to characterize the cellular localization of the IL-33 inthe rodent central nervous system (CNS). We have shown that IL-33 is present in the nuclei of oligodendrocyte lineage cells in the gray and white matter. Moreover, we demonstrated the changes in the IL-33 protein level associated with response of oligodendrocyte precursor cells, the largest undifferentiated cell population of adult CNS, to the injury. Our *in vivo* observations where verified by a series of in vitro experiments.

Our results suggest that IL-33 may be responsible for maintaining the constant pool of undifferentiated oligodendrocyte progenitors with regenerative potential.

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T02-13B

Characterization of the role of RET on enteric progenitors using Mosaic Analysis with Double Markers (MADM)

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The assembly of enteric neurons and glia into discrete ganglia and formation of functional neuronal circuits depends upon spatio-temporally regulated mechanisms that control the migration, proliferation and differentiation of ENS progenitors. Both enteric neurons and glia are derived from a pool of Sox10 expressing progenitors that have high neurogenic potential during early stages and a high gliogenic potential during late embryonic and postnatal stages of Enteric Nervous System (ENS) development. A key molecule that controls many aspects of ENS development is the receptor tyrosine kinase **RET**, which is expressed by undifferentiated multi-lineage progenitors and postmitotic enteric neurons. A series of genetic studies have established that Ret functions in both cell autonomous and non-cell autonomous manner. However, the role of Ret at single cell level has not been addressed.

To examine the effect of Ret deletion on individual ENS progenitors we used Mosaic Analysis with Double Markers (MADM). Using this strategy we have been able to differentially label daughter cells of single ENS progenitors with two different fluorescent markers - GFP and RFP. Taking advantage of the chromosomal location of the Ret gene on chromosome 6 in mice (distal to *Rosa26*, which has been targeted to generate the MADM alleles), we have been able to clonally delete Ret in GFP-expressing progenitors. This single-cell deletion resulted in a decrease in the neuronal population at

embryonic (E16) and early postnatal (P5) stages. This was followed by a total absence of $Ret^{-/-}$ neurons at adult (P30) indicating the importance of Ret for enteric neuronal survival. Interestingly, we also observed a reciprocal increase in the glial population at early postnatal stages (P5) and a distinct decrease in the number of encapsulating (Type I) glial cells in the ganglionated plexus and an associated increase of Type III glia in the non-ganglionated myenteric plexus. These data suggest a role of Ret signalling in the ratio of enteric neurons and glia and the different subtypes of enteric glial cells.

Our work establishes a genetic system which can be used to address the role of Ret signalling on ENS development at the single progenitor cell level and the role for Ret in enteric glial development.

T02-14B

Characterization of Chd7 expression and function in oligodendrogenesis and (re)myelination

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Oligodendrocytes are myelin-forming cells of the central nervous system wrapping axons and therefore permitting the saltatory conduction of action potentials. In Multiple sclerosis (MS), myelin sheath is destroyed and remyelination, that should normally occurred, is less and less efficient during disease progression. Therefore, a better understanding of OPC generation and differentiation is essential to develop more efficient remyelinating therapies. Oligodendrogenesis, involving the steps of generation (e.i. specification), differentiation and maturation of oligodendrocytes, is a mechanism controlled by specific transcription factors including Ascl1 and Olig2. Mutant mice (knockout) lacking either of these genes has a reduced formation and deficient differentiation of oligodendrocytes. How these transcription factors control oligodendrogenesis is still poorly unknown. Therefore, we have identify Ascl1/Olig2 common direct target genes by genome wide transcriptome comparisons of mutant mice and chromatin-immunoprecipitation (ChIP) for Ascl1 & Olig2, as one step toward the understanding of the mechanism involved in oligodendrogenesis. One of these targets identified, Chd7 (Chromodomain-Helicase-DNA-Binding 7), is a chromatin remodeling protein that can bind to methylated histones and add or remove nucleosomes, in order to open or close the chromatin. By immunofluorescence we demonstrate that Chd7 protein is specifically enriched in oligodendroglia. Chd7 is present in all OPCs but the strongest Chd7 levels are found in immature oligodendrocytes while its expression decline to lower levels in mature oligodendrocytes. Moreover, in a focal model of brain de/remyelination (lysolecitin), Chd7 expression is found preferentially in newly differentiating oligodendrocyte in/around the lesion. To assess the function of Chd7 in oligodendrogenesis, we are conducting loss-of-function in mice by conditional knockout ($Chd7^{flox}$ allele) at different stages. By inducing an OPC-specific Chd7 deletion in a time controlled manner ($PDGFR\alpha\text{-}CreER^{T}$; $Chd7^{flox}$; $Rosa26^{stop\text{-}Tom}$ mice), we are studying Chd7 function in oligodendrocyte differentiation and/or maturation. Our preliminary data suggest a strong requirement of Chd7 for oligodendrocyte differentiation during normal myelination. We are currently assessing Chd7 during remyelination both in the mouse focal demyelination (brain corpus callosum and spinal cord dorsal funiculus) and in different MS lesions.

T02-15B

Identification and characterization of distinct astroglia subpopulations in health and disease

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Astrocytes are the most abundant cell type in the central nervous system (CNS). Although numerous studies have elucidated the physiological importance of these cells, many biological aspect of astroglial remain unclear. Previously our group has shown that the glutamate-transporter GLT1/EAAT2 is almost exclusively expressed by astroglia. Aberrant expression of EAAT2/GLT1 has been shown to occur in or contribute to numerous neurological and psychiatric disorders, such as amyotrophic lateral sclerosis (ALS). Given the importance of astroglial glutamate transport to synaptic physiology, our original aim in this study was to explore the transcriptional regulation of the GLT1/EAAT2 promoter.

We generated mouse models that selectively express tdTomato- labeled promoter fragments of different sizes upstream of the GLT1 transcriptional start site. To our surprise, promoter lengths up to 7 kb lead to only neuronal expression in vivo. However, a promoter length of 8.3 kb, lead to specific expression of the tdTomato reporter to only astroglia (not neurons). Of all the astroglia, only a unique subset expressed tdTomato in the cortex, spinal cord, and cerebellum, but not hippocampus. We further crossed this model with BAC-GLT1-eGFP mice to label all GLT1-positive astroglia with eGFP. Using this double transgenic model as a tool to further characterize heterogeneous astroglia populations, we purified both tdTomato-positive and -negative astroglia using fluorescence-activated cell sorting (FACS). We then analyzed the transcriptome and proteome profiles using microarray and Isobaric tag labeling for relative and absolutely quantitation (iTRAQ) analysis, respectively. A list of candidate markers and pathways were generated and validated with quantitative PCR and immunohistochemistry. Of these candidate markers, one marker of interest was further explored using fate-map lineage tracing. This allowed us to elucidate its involvement in the development of this astroglia subpopulation. In addition, in order to explore the transcriptional regulation of the tdTomato fluorescence of these astroglia *in vivo* we performed live multiphoton microscopy of the mouse cortical layers I-IV. Lastly, we decided to explore the properties of these astroglia in a motor neuron disease context. In doing so, we used the BAC-GLT1 and tdTomato mice crossed to SOD1G93A and Thy1.2-TDP-43 mice respectively. We found that different subpopulations of astroglia are affected during disease progression. Taken together, these results shed light on the heterogeneous population of astroglia and the susceptibility of certain astroglia subpopulations to disease.

T02-16B

The PI3K/Akt inhibitor LY294002 induces astrogliosis in mouse cerebellar slices

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A key function of astrocytes is to respond to CNS damage by a classic reactive astrogliosis, characterised by proliferation and morphogenesis. Many extracellular factors are implicated in regulating reactive astrogliosis, but the underlying mechanisms are not fully elucidated. Here, we have examined the role of PI3K/Akt signalling, which regulates multiple intracellular pathways that are implicated in cell proliferation and differentiation. Mice aged postnatal day (P)12 were killed humanely in accordance with the Home Office Animals (Scientific) Act 1986 (UK) and brains were isolated to chilled artificial CSF for preparation of cerebellar slices and isolated optic nerves with retina intact. Cerebellar slices from GFAP-EGFP mice were maintained in organotypic culture for 7 days in vitro (DIV) in control medium or medium containing the PI3K/Akt inhibitor LY294002, which increased the number of astrocytes and had a marked effect on astrocyte morphology. This was examined further by microarray analysis of optic nerves, which identified connexin (Cx) 43, the major astrocytic gap junction protein, as one of the most altered genes following treatment with LY294002. The results provide evidence of a role for PI3K/Akt in regulating proliferation and morphogenic changes in astrocytes consistent with reactive astrogliosis and implicate Cx43 in these astroglial changes.

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T02-17B

Cell genesis and dendritic plasticity: a neuroplastic pas de deux in the onset and remission from depression

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The ability to set an appropriate response to stimuli through dynamic rearrangements of synapto-dendritic networks, as well as by regulating the generation of new neuronal and glial cells, renders the brain highly mutable. These phenomena, collectively known as neuroplasticity, are critical to promote the neuronal adaptations; its failure is now increasingly considered to be a major component in many neuropsychiatric conditions. Among these, depressive spectrum disorders are a paradigmatic example of the importance of neuroplastic alterations in the adult brain. A comprehensive picture of the effects of stress, a major trigger factor in depression, in the (de)regulation of neuroplasticity, including neuro- and gliogenesis, will be provided; the latter is, in turn, related to the emergence of physiological and behavioral alterations comprised in the symptomatic profile of depressive disorders. While these molecular and physiological mechanisms regulating neuroplastic processes are relevant for the onset of depressive symptoms, they also proved to be implicated in the action of antidepressants. So far, and although there is still much to be elucidated, it is becoming increasingly evident that the triad stress-neuroplasticity-depression constitutes ground for new findings and discoveries.

T02-18B

Nastructured interface promoting astrocytes molecular and functional differentiation in vitro

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Growing evidence indicated that the capability of astrocytes to act as homeostatic regulators in brain is related to patchy distribution of ion channels and aquaporins in plasma-membrane microdomains. Currently available *in vitro* models do not allow to reproduce the polarized expression of astroglial channels, which typically appear mislocalized in the cytosol as well as in the whole-plasma membrane, hampering the possibility to study at molecular level astroglial physiology in a in vivo-like model.

Nanostructured materials are a promising tool to control cell molecular and functional behavior through the nanoscale interaction with cell surfaces. Here, we investigate the adhesion, viability, molecular and functional properties of primary cultured rat neocortical astrocytes grown on hydrotalcite nanoparticles (HTlc NP) processed as films. Cell viability assay showed that HTlc-films supported astrocytes adhesion and survival with value comparable to those of Poly-D-Lysine (PDL). Morphological analyses showed that growth on HTlc NP films induced a starlike morphogy typical of differentiated astrocytes *in vivo*. Western blot (WB) analyses and immunofluorescence (IF) confocal imaging revealed that stellation was not accompanied by GFAP upregulation, indicating that differentiation was not due to occurrence of gliotic reaction. WB, IF, whole cell patch-clamp and water permeability measurement revealed that differentiation was accompanied by molecular and functional up-regulation and polarized membrane expression of both inward rectifying potassium channel Kir 4.1 and aquaporin 4, AQP4. The interaction between astrocytes and surface nanotopography was also investigated by Atomic Force Microscopy, Scanning Electron Microscopy and immunofluorescent analysis revealed an interaction of nanostructured interface with adhesion-mediating protein as well as reorganization of actin cytoskeleton.

Collectively, these results indicate that HTlc NP films are suitable substrates to generate novel *in vitro* models where to modulate, alter and study the specific functionality and expression of membrane channels of astrocytes.

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T02-19B

S100B modulates oligodendrocyte development process

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We recently observed that S100B is highly expressed in the CSF and brain samples of Multiple Sclerosis, while its neutralization in an ex vivo demyelination model prevents demyelination and glia reactivity. S100B is expressed by oligodendrocyte precursor cells (OPC) and reported as a crucial player in their differentiation, however high S100B levels released upon brain damage may be toxic for oligodendrocytes (OL). Thus, we intend to evaluate the effect of S100B on oligodendrocyte differentiation and migration.

Primary mixed glial cultures were obtained from rat cortices and OPC isolated after 10 days in vitro (DIV). OPC were incubated with 10 or 1000 nM S100B, to mimic the neurotrophic or toxic effects of S100B, respectively, for 24 h, just after isolation or in the first 24 h of differentiation. After 7 DIV cell were evaluated for OL differentiation and maturation and expression of differentiation-related Olig1. To study the role of S100B on OPC migration, cells were allowed to migrate to 20 ng/mL FGF for 24h, either in the presence of S100B (chemotaxis) or S100B plus FGF (chemokinesis) using the Boyden chamber.

Treatment of OPC with the 1000 nM S100B during proliferation resulted in a significant reduction of MBP+ cells (0.7-fold, $P<0.05$) with an increase of NG2+ cells (1.6-fold, $P<0.05$), while treatment during initial differentiation induced a less pronounced effect. To assess the effect of S100B on OL morphologic maturation, MBP+ cells were scored in: 1) cells with poorly branched, 2) cells with complex branched processes and 3) cells with complex branched processes that partially form membranes. Exposure of OPC to the 1000 nM S100B during proliferation increased the number of mature OL in stage 1 (1.6-fold, $P<0.05$), decreasing them in stages 2 and 3 (0.9-fold and 0.4-fold, respectively). These changes were augmented when OPC exposure occurred during differentiation (stage 1 1.9-fold, stage 2 0.8-fold and stage 3 0.1-fold, $P<0.05$). Moreover, treatment with 10 nM S100B during proliferation increased Olig1 (1.8-fold, $p<0.05$), while the highest concentration of S100B reduced Olig1 expression during differentiation, corroborating the reduced OPC differentiation toxic S100B treatment. Concerning migration, 1000 nM of S100B markedly impaired chemotaxis and chemokinesis ability (0.5-fold and 0.3-fold, $p<0.05$, respectively).

Overall, these results suggest that S100B affect oligodendrogenesis, being a potential target for new therapeutic approaches in myelin-related disorders.

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T02-20B

Overcoming age-dependent restriction of retinal müller glia in cell cycle re-entry

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Background: Adult mammalian retina have the potential to regenerate retinal neurons, but regenerated neurons are restricted at least due to limited number of Müller glia (MG) re-entering cell cycle and lack of progeny expansion. Here, we investigated animal age-dependence of the MG response upon retina damage and explored ways to stimulate MG proliferation and progeny generation.

Methods: Mouse retinas at various ages were damaged by *N-methyl-D-aspartate (*NMDA), and various factors were applied to stimulate MG proliferation in vivo. EdU was administered to label proliferating cells. Doxycycline was used to activate the cell cycle regulator CyclinD1 and Cdk4 (4D)

complex in hGFAP-cre::Rosa-rTA-ires-EGFP::tetO-4D transgenic mouse retina. Retinal wholemount or sections were analyzed.

Results: Brn3, NeuN and Calb2-positive neurons in the RGL decreased ~2.5-fold in NMDA- damaged retinas compared to undamaged control after 7 days post lesion (dpl). In EGF treated retina, at 7 dpl among 32 ±4 SEM total EdU+ cells per mm (N=3) 4 ±0.3 SEM were EdU+ Sox9+ double positive suggesting MG had re-entered the cell cycle. Interestingly, upon additional inhibition of TGFβ signaling total EdU+ cells (82 ±2 SEM per mm; N=3) and EdU+ Sox9+ cells (11 ±1 SEM per mm; N=3) increased ~2.5-fold compared to EGF. We observed the highest MG proliferative response in HB-EGF treated retinas (38 ±2 SEM EdU+ Sox9+ cells out of 177 ±10 SEM total EdU+ cells per mm; N=4), which was ~3.5-fold higher compared to EGF plus TGFβ inhibitor. Interestingly, the MG proliferation response decreased significantly with increasing animal age (P10: 38 ±2 SEM versus P12: 2.53+0.1 Sox9+EdU+ cells; N=4, p SEM RFP+ EdU+ cells per mm; N=4) upon 4D overexpression. And increased cell proliferation was confirmed by analyzing the expression of Ki67+ cells. 4D overexpression leads to ~5.4-fold increase in Ki-67+ cells per mm (364 ±8 SEM; N=4) compared to HB-EGF (67 ±3 SEM; N=4) treated retina.

Conclusion: Higher numbers of MG proliferation could be stimulated in juvenile retina compared to any previous report in the literature. Our data suggests the possibility of MG cell cycle re-entry and de-differentiation depend on animal age, appropriate mitogen stimulation and manipulation of microenvironment. Notably, increased expansion of MG can be induced genetically by overexpression of 4D complex upon neurotoxin-induced retina degeneration. Thus, it will be of great interest to find out (1) the fate of MG-derived progeny, and (2) the mechanisms that regulate and restrict Müller glia proliferation and stem cell competence.

T02-21B

Role of Dbx1 and Notch signalling in the specification of a subset of spinal astrocytes

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Studies of the last years have unravelled the high heterogeneity in morphology and function of astrocytes in central nervous system. However, there is still an incomplete understanding about the cellular and molecular control of their development. By using mouse genetics, we studied a small group of ventral progenitors of the embryonic spinal cord -called p0-, which expresses the transcription factor Dbx1. After the production of the motor-related V0 Interneurons (V0-IN), this progenitor pool is later committed to generate a group of glial cells that we identify as astrocytes and named as “vA0” (ventral astrocytes from p0 domain). vA0 precursors begin to leave the ventricular zone at E14.5 and follow a stereotyped radial migratory pathway, probably through nuclear translocation, to populate a defined region of the lateral spinal cord. Mosaic fluorescent labelling showed that vA0 population is composed by both protoplasmic and fibrous astrocytes, demonstrating that a single progenitor domain produces astrocyte with different morphological features. We found that Dbx1 controls specification of vA0 astrocytes, as in its absence vA0 is augmented at the expense of V0-INs, which were born earlier. Notch signalling plays key roles in binary fate-cell decisions and glial determination. We evaluated if this pathway modulates early decision between producing V0 neurons and vA0 astrocytes, and whether differential Notch activity could be involved in Dbx1 function. Presenilin1 mutants, that have severely attenuated Notch signalling, exhibited diminished p0-derived glial cells, while V0 INs numbers were increased. Impairing Notch pathway with Ly411575 only at neurogenic stages, but not later, showed similar results. Additionally, gliogenic precursors are increased in Dbx1 mutants and are reduced in Psen1 mutants after the neurogenic stage. This prompted us to analyze key players of Notch signalling pathways in Dbx1 wt and mutant neural tubes before the initiation of gliogenesis. Surprisingly, while wild type p0 domain expresses Delta1 ligand, this domain is converted into Jagged1 positive in the absence of Dbx1. Our results suggest that the type of Notch ligand directed by Dbx1 transcription factor controls the differentiation of p0 precursors by biasing neurogenic vs astrogenic fate.

T02-22B

Genetic ablation of proliferating NG2-glia in the adult brain

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NG2-glia - also known as oligodendrocyte progenitor cells- constitute a cell fraction of ~5% of the brain, are the only proliferating cells in the adult brain parenchyma and generate myelinating oligodendrocytes throughout lifetime. Despite their high number and substantial characterization, their role in the intact and injured adult brain remains unclear. To address this, we used conditional genetic deletion of the Esco2 protein in the inducible Sox10iCreER^{T2}xCAG-eGFPxEsco2$^{fl/fl}$ mouse line that triggers apoptosis specifically of dividing NG2-glia during M-phase. We could show that deletion of Esco2 in NG2-glia in the adult brain induced progressive cell death in the dividing recombined cells that was compensated by enhanced proliferation of the non-recombined ones. Specifically in the white matter of the cerebral cortex, we observed a decreased number of newly generated oligodendrocytes followed by structural changes in the nodes of Ranvier. Interestingly, these animals -in contrast to control littermates- developed progressive motor deficits.

As proliferation of NG2-glia is >15-fold increased after stab wound injury, we analyzed here the role of NG2-glia by ablating them after acute lesion. We found a reduction of NG2-glia around the lesion site and are currently analyzing their role in the injury progression and closure. Our data suggest that NG2-glia and the newly generated oligodendrocytes in the adult brain are important for the maintenance of myelin associated structures in the intact brain as well as for the injury related glial reaction in the pathological brain.

T02-23B

Chimeric OHSCs as culture system to study microglia phenotypes

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Microglia, the resident macrophages of the brain, can adopt a variety of phenotypes, ranging from a tolerant, immune-suppressed phenotype to a primed, hypersensitive phenotype. Microglia display a tolerant phenotype after an inflammatory challenge[1], while the primed phenotype is observed during ageing and neurodegenerative conditions[2]. Microglia are critical to maintain homeostasis of the brain, and at present it is unclear how these diametrically opposed phenotypes affect normal microglia function and neuronal support. Tolerant microglia are immune suppressed but display enhanced phagocytic activity, suggesting a more inflammation resolving phenotype that might actually protect tissue to excess damage in case of repeated inflammatory challenges. On the other hand, the hypersensitive state of primed microglia may lead to an increase in cellular damage in reaction to homeostatic disturbances and could contribute to neurodegenerative diseases.

To determine the physiological consequences of tolerant versus primed microglia, we describe here an organotypical hippocampal slice culture (OHSC) system[3]. In OHSCs, neuronal tissue architecture remains well preserved and is accessible for manipulation and cell replacement strategies. To study the function of different microglia phenotypes, chimeric OHSCs were generated. In short, the endogenous microglia were depleted by applying clodronate to OHSCs and replenished with either tolerant or primed microglia. The source of primed microglia are 2 months old ERCC1-deficient mice, a mouse model for accelerated ageing[4]. Tolerant microglia were isolated from mice injected with Lipopolysaccharide (LPS). Two weeks after replenishment the chimeric OHSC were analyzed using both qPCR and staining. The analysis show that the phenotype of microglia remains stable. Here we present the chimeric OHSC model as an useful model to study the effects of different microglia phenotypes on the surrounding tissue.

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Poster topic 03
Cell signaling

T03-01A

Activated Microglia/Macrophage Whey Acidic Protein (AMWAP) inhibits NFκB signaling and induces a neuroprotective phenotype in microglia

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Background: Microglia reactivity is a hallmark of degenerative diseases of the brain and the retina. We have previously identified Activated Microglia/Macrophage Whey Acidic Protein (AMWAP) as a counter-regulator of pro-inflammatory response. Here, we studied its mechanisms of action with a focus on Toll-like receptor (TLR) and nuclear factor κB (NFκB) signaling and tested its neuroprotective effects.

Methods: Recombinant AMWAP was produced in E. coli and HEK293 cells and purified by affinity chromatography. AMWAP uptake was identified by fluorescent labeling and pro-inflammatory microglia markers were measured by qRT-PCR after stimulation with TLR ligands. NFκB pathway proteins were assessed by immunocytochemistry, Western blot and immunoprecipitation. A 20S proteasome activity assay was used to investigate the anti-peptidase activity of AMWAP. Microglial neurotoxicity was estimated by nitrite measurement and quantification of caspase 3/7 levels in 661W photoreceptors cultured in the presence of microglia-conditioned medium. Microglial proliferation was investigated using FACS and their phagocytosis was monitored by the uptake of 661W photoreceptor debris.

Results: AMWAP was secreted from lipopolysaccharide (LPS)-activated microglia and recombinant AMWAP reduced gene transcription of IL6, iNOS, CCL2, CASP11, and TNFα in BV-2 microglia treated with LPS as TLR4 ligand. This effect was replicated with murine embryonic stem cell derived microglia (ESdM) and primary brain microglia. AMWAP also diminished pro-inflammatory markers in microglia activated with the TLR2 ligand zymosan, but had no effects on IL6, iNOS, CCL2 and TNFα transcription in cells treated with CpG oligodeoxynucleotides as TLR9 ligand. Microglial uptake of AMWAP effectively inhibited TLR4-dependent NFκB activation by preventing IRAK-1 and IκBα proteolysis. No inhibition of IκBα phosphorylation or ubiquitination and no influence on overall 20S proteasome activity were observed. Functionally, both microglial nitric oxide (NO) secretion and 661W photoreceptor apoptosis were significantly reduced after AMWAP treatment. AMWAP promoted filopodia formation of microglia and increased the phagocytic uptake of apoptotic 661W photoreceptor cells.

Conclusions: AMWAP is secreted from reactive microglia and acts in a paracrine fashion to counter-balance TLR2/TLR4-induced reactivity through NFκB inhibition. AMWAP also induces a neuroprotective microglial phenotype with reduced neurotoxicity and increased phagocytosis. We therefore hypothesize that anti-inflammatory whey acidic proteins could have a therapeutic potential in neurodegenerative diseases of the brain and the retina.

T03-02A

Prenatal stress alters microglial and inhibitory neuron development in an animal model of infantile spasms

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Infantile spasms (IS), are an age-dependent epileptic encephalopathy with severe cognitive dysfunction. In recent years, numerous studies have found that prenatal stress to increases the risk of IS. However, there is still more research to be done to provide a profound understanding of how prenatal stress affects GABAergic progenitors and microglial development and distribution in the postnatal brains.Here, we developed a model of spastic seizures triggered by N-methyl-D-aspartate in infant rats which were prenatally exposed to betamethasone and acute immobilization stress. Firstly,Prenatal stress reduced the number of immature microglia and promoted an accelerated microglial differentiation into a ramified form.Secondly, we evaluated the developmental expression of glutamic acid decarboxylase isoforms 67 (GAD67)−the rate-limiting step of GABA synthesis−after exposure to prenatal stress from embryonic day 15. The distribution of GAD67 immunopositive cells with the superficial tangential migratory pathway was decreased after prenatal stress. Also, the density of GABAergic progenitors was reduced in the cortical plate of prenatally stressed rats at P16. Finally, we also examined the GABA excitatory/inhibitory shift, which is determined in part by a sequential development of two major chloride co-transporters, NKCC1 and KCC2. Prenatal stress considerably decreased the expression of KCC2 and increased NKCC1 expression compared to normal cortex. These results demonstrate that early disruption in GABAergic interneuron migration and delayed GABA excitatory/inhibitory shift caused by prenatal stress may enhance susceptibility to IS.

T03-03A

Involvement of transcription factors NF-κB, AP-1 and STAT-3 in death of crayfish glial and neuronal cells induced by photodynamic impact

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Photodynamic therapy (PDT) based on photogeneration of highly cytotoxic singlet oxygen, oxidative stress and following cell death is used for treatment of brain tumors. However, PDT damages not only malignant, but also healthy neurons and glial cells. We used activator or inhibitorsof transcription factors NF-κB, AP-1 and STAT-3 in the study of their potentialrolein photodynamic damage of isolated crayfish neurons and satellite glial cells. Alumophthalocyaine photosens was used as a photosensitizer; the laser diode (670 nm) was a light source. PDT-induced apoptosis of glial cells was increased under activation ofNF-κB bybetulinic acid, or decreased it the presence ofNF-κB inhibitorcaffeic acid phenethyl ester (CAPE), AP-1 inhibitor SR11302 or STAT-3 inhibitor stattic. Therefore,NF-κB, AP-1 and STAT-3were involved in PDT-induced apoptosis of glial cells. Necrosis of glial cells was reduced by betulinic acid and stattic, but enhanced by parthenolide. Therefore,NF-κB participated in protection of glial cells from PDT-induced necrosis, whereas STAT-3 played the pro-necrotic role. Necrosis of neurons was increased in the presence of betulinic acid, but reduced by CAPE and stattic, thereby indicating the involvement of NF-κB and STAT-3 in PDT-induced necrosis of neurons. Inhibition of AP-1 by SR11302 did not influence PDT-induced necrosis of neurons and glia.These data indicate the difference in signaling pathways mediated byNF-κB, AP-1 or STAT-3in crayfish neurons and satellite glial cells. Inhibitors of these transcription factors may protect normal neurons and glial cells from photodynamic injury. Supported by grants of Russian Foundation for Basic Research (14-04-00741), Russian Scientific Foundation (14-15-00068) and Russian Ministry of Education and Science (Research organization #790).

Table

Effect of modulators of activity of transcription factors NF-κB, AP-1 и STAT-3 on PDT-induced death of neurons and glial cells. ▲ - increase ($p<0.05$); ▼ - decrease ($p<0.05$); — no effect.

Modulator	Apoptosis of glial cells	Necrosis of glial cells	Necrosis of neurons
NF-κB			
Betulinic acid,5 μM (activator)	▲	▼	▲
Parthenolide, 20 μM (inhibitor)	—	▲	—
CAPE, 30 μM (inhibitor)	▼	—	▼
AP-1			
SR11302, 1 μM (inhibitor)	▼	—	—
STAT-3			
Stattic, 10 μM (inhibitor)	▼	▼	▼

T03-04A

Growth differentiation factor 15 (GDF15) expression in astrocytes after excitotoxic lesion in the mouse hippocampus

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Growth differentiation factor 15 (GDF15) is a member of the transforming growth factor β (TGF-β) superfamily of proteins. Although GDF15 is well established as a potent neurotrophic factor for neurons, little is known about its role in glial cells under neuropathological conditions. We monitored GDF15 expression in astrocyte activation after a kainic acid (KA)-induced neurodegeneration in the mouse hippocampus. In control, GDF15 immunoreactivity (IR) was evident in the neuronal layer of the hippocampus; however, GDF15expression had increased in activated astrocytes throughout the hippocampal region at day 3 after the treatment with KA. Because NF-kB plays an essential role in the expression of proinflammatory mediators by LPS, we next examined whether GDF15 influences LPS-induced NF-kB activity in astrocytes. Pretreatment of the astrocytes with GDF15 siRNA resulted in the inhibition of LPS-induced degradation of IkB-a; GDF15 siRNA also suppressed LPS-induced phosphorylation of RelA/p65 in these cells. These results indicate that GDF15 silencing suppresses NF-kB activity in the LPS-stimulated astrocytes through the inhibition ofkinase activity of the IKK complex. These results also indicate that GDF15 silencing has a potential to inhibit IKK-mediated NF-kB activation, making GDF15a valuable target for modulating inflammatory conditions.

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Keywords: GDF15, Astrocyte, Excitotoxicity, NF kappaB signaling

T03-05A

Calcium-induced calcium release and gap junctions mediate large-scale calcium waves in olfactory ensheathing cells *in situ*

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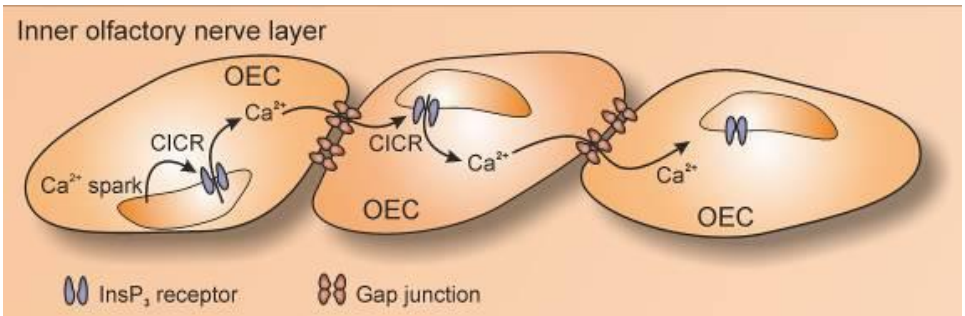
Olfactory ensheathing cells (OECs) are a specialized type of glial cells, supporting axon growth and guidance during development and regeneration of the olfactory nerve and the nerve layer of the olfactory bulb. We measured calcium signalling in OECs in olfactory bulb *in-toto* preparations using confocal and epifluorescence microscopy and the calcium indicator Fluo-4. We identified two subpopulations of olfactory bulb OECs: OECs in the outer sublamina of the nerve layer responded to purinergic neurotransmitters such as adenosine triphosphate with calcium transients, while OECs in the inner sublamina of the nerve layer did not respond to neurotransmitters. However, the latter generated spontaneous calcium waves that covered hundreds of cells. These calcium waves persisted in the presence of tetrodotoxin and in calcium-free saline, but were abolished after calcium store depletion with cyclopiazonic acid or inositol trisphosphate receptor blockage with 2-APB. Calcium waves could be triggered by laser photolysis of caged inositol trisphosphate. Blocking purinoceptors with PPADS had no effect on calcium wave propagation, whereas blocking gap junctions with carbenoxolone or meclofenamic acid entirely suppressed calcium waves. Increasing calcium buffer capacity in OECs with NP-EGTA ("caged" Ca^{2+}) prevented calcium wave generation, and laser photolysis of NP-EGTA in a small group of OECs resulted in a calcium increase in the irradiated cells followed by a calcium wave. We conclude that calcium waves in OECs can be initiated by calcium-induced calcium release via $InsP_3$ receptors and propagate through gap junctions, while purinergic signalling is not involved.

Image



T03-06A

Protein Tyrosine Phosphatase Alpha (PTPa)-mediated Akt activation is required for oligodendrocyte differentiation and myelination

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Oligodendrocytes (OLs) are the myelinating cells of the central nervous system. The myelination process is preceded by molecular and morphological differentiation of oligodendrocyte precursor cells (OPCs) into mature myelinating OLs. PTPa is a brain-enriched tyrosine phosphatase that regulates many cellular processes, including OPC differentiation. PTPa null (KO) OPCs have impaired differentiation and brains of KO mice are hypomyelinated. In our current study, we recapitulated this defect in organotypic cerebellar slice cultures derived from neonatal wild-type (WT) and KO mice. Slices were cultured for 10 and 20 DIV and immunostained for axons (NFH), OLs (MBP), and paranodes (Caspr). This revealed that KO cerebellar slices have impaired myelination as indicated by reduced MBP/NFH co-localization and formation of myelin-directed paranodes. We also observed defective myelination in neuron/OL co-cultures where WT and KO OPCs were introduced to neurite beds formed by dorsal root ganglion neurons for 14 days and immunostained for MBP and NFH. Indeed, MBP/NFH co-localization is significantly reduced by ~50% in co-cultures with KO OPCs as

compared to WT OPCs. This is coincident with a reduction in MBP signal from KO OPCs, indicating a differentiation defect in the absence of PTPa.

We are investigating how PTPa-dependent signaling regulates OPC differentiation. We find that Akt is activated in differentiating WT OPCs, but not in KO OPCs, as indicated by increased phosphorylation of Akt^{Ser473} and Akt substrates. In particular, phosphorylation of the Akt substrate NDRG1 was detected in differentiating WT OPCS but not KO OPCs. NDRG1 is known to play critical roles in myelination in the peripheral nervous system. Our findings indicate that NDRG1 may also be involved in OL differentiation that is prerequisite for CNS myelination. Our future studies will elucidate the signaling mechanisms by which PTPa regulates Akt/NDRG1 to promote these processes. Improved knowledge of the molecular pathways orchestrating OL development will provide insights to developing novel, effective, and specific treatments to promote myelin repair in demyelinating diseases.

T03-07A

An interactive model of astrocyte in 3D geometry

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Electrically non-excitable astrocytes appear competent in transducing, integrating and propagating physiological calcium signals. Decoding this type of signalling, however, poses a conceptual challenge because it requires an understanding of molecular interactions and Ca2+ dynamics in the massive morphological structure of nanoscale-thin leaf-like processes which constitute the bulk of astrocyte geometry. How a particular calcium signalling engages a particular type of local geometry remains therefore poorly understood.
There have been no attempts to develop an astrocyte model with such a complex morphology even though this could provide the key to mechanistic insights into astrocytic physiology and Ca2+ signalling.
To understand the role of complex shape in a cell function we have adapted the NEURON modelling environment to build a generic astrocyte model which can reproduce quantitatively the detailed morphology, membrane properties and known molecular signalling mechanisms of the typical astrocyte. The model enables distributed Ca2+ homeostasis mechanisms including diffusion, wave propagation, gap-junction escape or channel currents whereas the simulation environment has also the capability to mimic uncaging, membrane physiology, volume current injections or fluorescence recovery after photobleaching (FRAP) experiments in the 3D tissue volume containing the astrocyte. In our case study, the model has adapted the features of hippocampal protoplasmic astrocytes (area CA1) documented through a combination of experiments involving electrophysiology, two-photon excitation imaging, a FRAP super-resolution technique and quantitative electron microscopy. We demonstrate how simulations with the model could help to unveil some fundamental features of astrocytic morphology and Ca2+ signalling that are not accessible to direct experimental probing. To our knowledge, this is the first attempt to have a full-scale generic model of astroglia, which we believe will attract significant interest among a wide audience of cell biologists and neuroscientists.

T03-08A

The role of DNA methylation and histone deacetylation in reactions of glial cells to photodynamic treatment

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Photodynamic therapy (PDT) is used for cancer treatment, including brain tumors. But it is important to study the mechanisms of photooxidative damage of surrounding normal nervous tissue. Epigenetic processes are involved in the regulation of diverse cell functions and survival, but their role in oxidative

damage of neurons and glial cells are poorly understood. We studied the role of DNA methylation and histone deacetylation in responses of the nerve cells and glia to PDT. Isolated crayfish stretch receptor consisting of a single mechanoreceptor neuron and satellite glial cells is a simplest neuroglial preparation. It was photosensitized with alumophthalocyanine Photosens and irradiated by the diode laser (670 nm). PDT induced necrosis of a neuron and glial cells and glial apoptosis. Inhibitors of DNA methylation 5-aza-cytidine and decitabine suppressed PDT-induced necrosis of glial cells, but not neurons, by a factor of 1.3 and 2.0, respectively. It did not influence apoptosis of glial cells. Inhibitors of histone deacetylase valproic acid and trichostatin A inhibited PDT-induced necrosis and apoptosis of glial cells but not neurons, by a factor of 1.6-2.7. Thus, in the isolated crayfish stretch receptor DNA methylation and histone deacetylation were involved in the epigenetic regulation of necrosis of glial cells but not neurons. Histone deacetylation was also involved in apoptosis of glia surrounding mechanoreceptor neuron. Supported by grants of RFBR (14-04-00741), RSF (14-15-00068) and Russian Ministry of Education and Science (Research organization No 790).

T03-09A

Astrocytic expression of CTMP following an excitotoxic lesion in the mouse hippocampus

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The PKB (protein kinase B, also known as Akt) is a serine/threonine-specific protein kinases that plays a key role in multiple cellular processes, such as apoptosis, cellular survival, cell proliferation and transformation. PKB is activated by phosphorylation on residues threonine 308, by the protein kinase PDK1 (Phosphoinositide dependent kinase 1), and Serine 473, by a putative serine 473 kinase. Besides PKB activators, PKB is negatively regulated by endogenous inhibitors which serve as brakes on PKB signaling. The Carboxyl-Terminal Modulator Protein (CTMP) is a novel binding partner and endogenous inhibitor, which binds the carboxyl-terminal regulatory domain of PKB at the plasma membrane and suppresses the phosphorylation and activation of PKB. Although few studies have investigated the roles of CTMP, a negative regulator of PKB/Akt and relationship with multiple cellular function, little is known about CTMP changes in glial cell under neuropathological conditions. In this study, we evaluated the expressions of CTMP and effect on PKB, following the induction of an excitotoxic lesion in mouse brain by kainic acid (KA) injection, which caused pyramidal cell degeneration in the hippocampal CA3 region. In injured hippocampal CA3 region, CTMP was increased in astrocytes day 3 after treatment. However, phospho-CTMP was increased in astrocytes day 1 early than that of CTMP. For the first time, our data demonstrate the injury-induced astrocytic changes in the levels of CTMP in vivo, which may reflect mechanisms of glial cells protection or adaptive response to damage.

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T03-11A

Effect of long-term culture on telomere length and telomerase activity in murine brain microglia

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Microglia are the immune cells of the central nervous system (CNS) and play a fundamental role in supporting neuronal function and survival under both physiological and pathological conditions. Following acute CNS injury, there is rapid activation of microglia, marked by a conspicuous mitotic response. Like neurons, microglia also show age-related changes that can potentially alter their behaviour and contribute to the cognitive decline associated with aging. One hallmark of aging is shortening of telomeres, which in mitotic cells leads to their senescence. Telomere length is being used as both an in vitro and in vivo marker of cell replication and cell aging. Continual shortening of

telomeres is partially compensated by the ribonucleoprotein enzyme telomerase, which adds tandem repeats to 3' ends of mammalian telomeres and maintains genome integrity and stability. To evaluate the possibility whether continuous proliferation of microglial cells can induce replicative senescence in these cells in the mouse brain, we investigated the telomere shortening and telomerase activity in isolated microglia in vitro.

We developed a long-term mixed microglia/astrocyte culture, prepared from brains of newborn C57BL/6J mice by enzymatic digestion and mechanical trituration. Mixed cultures were splitted every two weeks until no significant proliferation was observed in viable cells. Microglia were isolated by shaking or mild trypsinization to yield pure cell populations. Relative telomere length in isolated microglia was determined with quantitative real - time PCR and telomerase activity by means of the telomeric repeat amplification protocol (TRAP) assay.

In our new protocol, viable microglia continuously proliferated up to 33 days in mixed cultures, with a steady decline in the proliferation rate starting on day 18. Microglia isolation by shaking and mild trypsinization yielded 96 and 98% of pure cells respectively. Relative telomere length was significantly reduced by 27% between day 14 and day 35 in culture and correlated with a 5-fold increase in telomerase activity in the same period of time.

This study shows that murine brain microglia become senescent in vitro, as indicated by a reduced proliferation rate, telomere shortening and an increase in telomerase activity.

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T03-12A

$P2X_7$ receptor stimulation in the presence or absence of calcium leads to antagonic signaling pathways activation in neurons

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$P2X_7$ belongs to the family of ionotropic ATP-gated receptors, which function as ion channels mediating calcium influx. $P2X_7$ is one of the predominant purinergic P2X receptor subtype expressed in neural cells. It is involved in a variety of processes such as ATP-mediated cell death, regulation of receptor trafficking, and inflammation. It is largely known that $P2X_7$ receptor stimulation leads to regulation of the mitogen-activated protein kinases (MAPK) extracellular regulated kinases (ERK1/ERK2), c-Jun N-terminal kinase (JNK) and p38. The canonical pathway for the activation of these MAPKs is mediated by Ras.

Here, we show that $P2X_7$ receptor stimulation triggers not only the MAPK activation but also the Ras GTPase activation. Besides, we demonstrate that in the presence of calcium this Ras/MAPK pathway regulation is mediated by the Ras guanine exchange factor (GEF) RasGRF1. For its activation, this GEF requires a calcium input from the extracellular medium, which will intracellularly bind to calmodulin. In this configuration, Ca^{+2}-loaded calmodulin binds to the RasGRF1 IQ motif and activates it. Surprisingly, $P2X_7$ also activates Ras in a Ca^{+2}-independent manner. In addition, this alternative Ras activation leads to the activation of the small GTPase Rap1 and more importantly, triggers neural cell death. Our findings demonstrate that $P2X_7$ stimulation in neurons activates two different signaling pathways depending on the presence or absence of extracellular Ca^{+2}, leading to cell survival *versus* cell death.

T03-13A

Integrin b1 triggers amyloid b-induced astrocyte reactivity through NOX2 activation in Alzheimer disease models

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Astrocyte reactivity is a hallmark of Alzheimer disease (AD) and can constitute a primary pathogenic element of the disease. Elucidation of the signaling cascades inducing reactivity in astrocytes during AD would help characterizing the function of these cells and identify novel molecular targets to modulate disease progression. Here, we describe a novel mechanism by which the toxic soluble amyloid beta (Ab) modulates integrin beta 1 activity and triggers a reactive oxygen species (ROS)-dependent astrogliosis *in vitro* and *in vivo*. First, we observed that Ab oligomers induce ROS production which is prevented by NADPH oxidase (NOX) inhibitors, and that oligomers differentially regulate NOX2 and NOX1 mRNA and protein levels, in a ROS dependent manner. To further investigate the pathway underlying Ab-mediated ROS generation, we analyzed the activation of NOX-interacting protein Rac by Rac-GTP affinity precipitation and PAK1 phosphorylation assay. We found that Ab oligomers produce a sustained Rac activation that is blocked by inhibitors of the classic but not by the novel PKC activities and also by wortmannin, a PI3K inhibitor. Moreover, Ab-induced ROS generation is reduced by an antibody against integrin beta 1, suggesting that this protein is upstream of PI3K/PKC/Rac/NOX pathway activation. Importantly, Ab oligomers induce GFAP, integrin b1 and NOX2 overexpression mediated by integrin b1 activation and NOX-dependent signaling in cultured astrocytes. These findings were confirmed using *in vivo* models. Intrahippocampal injection of amyloid b oligomers overexpressed astrogliotic and oxidative stress markers (GFAP, S100, grp78 and NOX2) that were reduced by coinjection of Ab-oligomers with the antibody against integrin b1. These data were validated in a triple-transgenic mouse model of AD and in postmortem brains of individuals with AD. Biochemical analysis showed that dysregulation of GFAP, NOX2 and integrin b1 levels correlated with Ab oligomers accumulation in vivo. Furthermore, we found that integrin b1 and NOX2 levels were significantly higher in reactive astrocytes in triple-transgenic mice than in wildtype mice, as well as in AD brains as compared to controls. These data suggest that Ab oligomers may directly cause and exacerbate astrocyte reactivity in AD by enhancing integrin b1 and NOX2 activity via ROS-dependent mechanisms. These observations may be relevant to AD pathophysiology.

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T03-14A

Primary radial glial cell culture as a model for dopaminergic regulation of neuroestrogen synthesis

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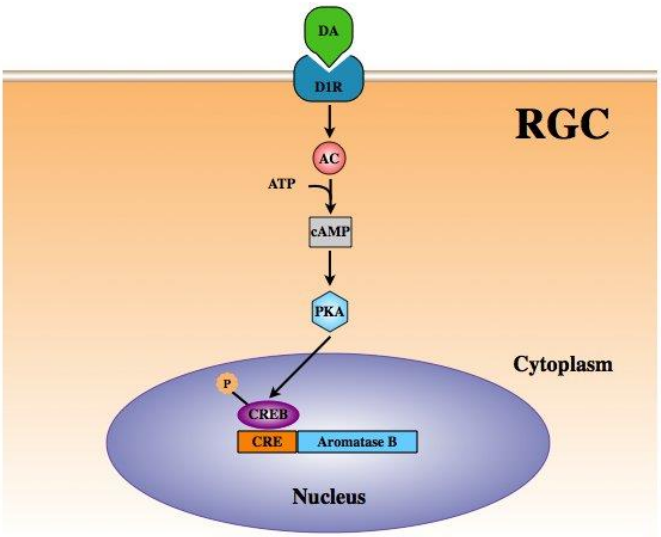
Estrogens produced locally in the vertebrate brain play many fundamental roles in differentiation, neural regeneration, neuroendocrine functions, reproductive functions and socio-sexual behaviours. Radial glial cells (RGCs) are neuronal precursor cells that are very abundant in fish brains and are the exclusive site of aromatase B expression and estrogen synthesis from androgen precursors. Using in vivo approaches coupled to a novel in vitro cell culture preparation we address the important questions of whether RGCs are capable of de novo steroid synthesis from cholesterol and what regulates neuroestrogen synthesis. We found a close anatomical relationship between RGCs and tyrosine hydroxylase positive catecholaminergic neurons along the telencephalon ventricular surface. Immunofluorescence analysis indicates that cultured RGCs from female goldfish retain their normal in vivo characteristics. More than 95% of the RGCs co-express glial fibrillary acidic protein and brain lipid binding protein. Gene cloning and sequencing revealed the presence of steroidogenic acute regulatory protein, and the key cytochrome P450 steroidogenic enzymes in cultured RGCs, indicting the potential of RGCs to produce numerous steroids in addition to estrogens. As determined using gene cloning and immunocytochemistry, RGCs express dopamine receptor (D1R). Pharmacological experiments established that activation of the D1R up-regulates aromatase B by a cAMP/PKA/CREB-dependent mechanism. These emerging neuroanatomical and gene expression data indicate that RGCs express

steroidogenic enzymes and that neurotransmitters in neighboring neurons can regulate neuroestrogen synthesis. Other anatomical data suggest that RGCs may also communicate with catecholaminergic neurons. While the exact functional significance of dopaminergic regulation or aromatase remains firmly established, these data have major implications for our understanding neurogenesis given the known roles of RGCs and estrogens in this fundamental process.

Image



T03-15A

The role of CD200R/Foxp3 signaling as enhancer of alternative activation of microglia

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The main role of the immune system is to protect the organism against damage by ensuring an adequate response against various pathogens. The immune system, therefore, has to comprise homeostatic mechanisms, including both activatory and inhibitory function. The interaction of CD200 with its receptor CD200R plays a significant role in maintaining microglia in a quiescent state. However, the role of CD200/CD200R interaction during alternative activation of microglia has not been described yet. Foxp3 is one of the most important transcriptional repressor and inhibits proinflammatory system. We examined the role of CD200-CD200R with Foxp3 on alternative activation of microglia in kainic acid (KA)-induced mouse hippocampus. Foxp2 expression is upregulated in microglia cells following in KA-induced excitotoxicity. Fopx3 is up-regulated in alternative activation induced by IL-4 of microglia and is dependent on CD200-CD200R signaling. Anti-inflammatory cytokine is up-regulated in alternative activation of microglia through CD200-CD200R signaling and Foxp3. Taken together, it suggests that Foxp3 may regulate alternative activation of microglia through CD200/CD200R interaction.

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T03-16A

ER stress induces autophagy impairment in the spinal dorsal horn in a model of neuropathic pain

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Endoplasmic reticulum (ER) stress has been implicated in neurodegenerative disease but its role in neuropathic pain remains unclear. In this study, we examinedthe association of ER stress and the unfolded protein response (UPR) with autophagic activity in a L5 spinal nerve ligation (SNL)-induced neuropathic pain rat model. SNL-induced neuropathic pain was assessed behaviorally, using a CatWalk system, and histologically, by quantifying microglial activation in the dorsal spinal horn. Among UPR sensor proteins, expression of BIP and ATF6 were increased in spinal dorsal horn neurons. Spliced XBP1 was also significantly elevated in the ipsilateral spinal dorsal horn. The PERK-eIF2 pathway was activated in astrocytes of the SNL model spinal dorsal horn. LPS-treated microglia conditioned medium induced ER stress and autophagic activity in cultured neurons through the ATF6 and IRE1-XBP1 pathways, but not the PERK-eIF2 pathway. Electron microscopy revealed swollen cisternae and autophagosomes in the dorsal spinal cord after SNL. Inhibition of the ATF6 pathway by intrathecal treatment with ATF6 siRNA reduced pain behavior and autophagic activity. This suggests that an accumulation of autophagic markers in response to immune-mediated ER stress might be involved in the induction and maintenance of neuropathic pain. Furthermore, a disturbance of autophagic signaling may render spinal neurons vulnerable to peripheral nerve injury or neuropathic pain stimuli.

Acknowledgements

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T03-17A

Analysis of the expression of the wnt family of proteins in activated astroglial cells

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Under CNS pathological conditions, activated astroglial cells develop a complex and versatile response, comprising a plethora of cellular changes and allowing their involvement in many beneficial and deleterious processes that profoundly influence tissue and cell viability. Hence, a great effort has been done to shed light on the molecular mechanisms that regulate astroglial activation and mediate the effects exerted by this cell type. Interestingly, the Wnt family of proteins has recently emerged as a pivotal regulator of many of the biological processes that characterize the progression of different neuropathologies, including the astroglial activation. However, our knowledge about the expression of this family of proteins in astroglial cells is scarce. Thus, the aim of this work is to evaluate the expression of the components of the Wnt family of proteins in non-activated and activated astroglial cells. For this purpose, pure cortical astroglial cultures were treated or not with LPS (100 ng/ml) or a protein extract obtained from contused spinal cords (SC) (200 µg/ml). Astroglial cultures were then processed for: (1) GFAP immunocytochemistry to evaluate cell number and area, (2) Real Time PCR to analyze the expression of prototypical markers of astroglial activation and the components of the Wnt family of proteins, and (3) Western Blot to evaluate the activation state of the three actually known Wnt-dependent signaling pathways, the Wnt/β-catenin, the Wnt/JNK and the Wnt/Ca2+ pathways. Firstly, our results demonstrate that, although we did not detect variations in cell area and number, both LPS and SC induced changes in the expression of prototypical markers of astroglial activation, even displaying intriguing differences between both activation systems. Moreover, here we show that most Wnt ligands, receptors and modulators were expressed in non activated astrocytes, and that after

activation the expression of the majority of these molecules suffered evident changes, which were both dependent and independent of the activation method. Finally and concomitant with the previously detailed observations, we also detected activation-dependent changes in the three Wnt-dependent signaling pathways. In conclusion, here we demonstrate that, under basal conditions, astroglial cells express many of the components of the Wnt family of proteins, whereas their activation induces evident changes in their expression patterns, suggesting that these molecules are involved in the astroglial response to injury.

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T03-01B

Thyroid hormone and AMPc/PKA pathway play a role in the elongation of oligodendroglial processes

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We have already showed the importance of thyroid hormone (T3) in oligodendroglial differentiation, its relationship with cytoskeleton proteins and its role in myelin proteins distribution such as CNPase and MBP. We have also showed that MAPK/ERK pathway has a role in oligodendrocyte processes elongation and branching. The AMPc/PKA pathway may be also relevant for oligodendroglial morphology. PKA can inhibit RhoA and consequently increases the process elongation. In this study we aim to evaluate the relationship between T3 and AMPc/PKA signaling on RhoA distribution and oligodendroglial morphology, *in vitro*. Oligodendroglial cells were obtained from cerebral hemispheres of newborn rats and cultured with or without T3 (T3 or -T3) in the medium. After 5 days these two groups were divided accordingly to each drug treatment: DMSO (control); an adenylyl cyclase activator - forskolin (FORSC) [10 mM]; an adenylyl cyclase inhibitor - SQ22356 (SQ) [1 mM]; and an inhibitor of PKA, H-89 [1 mM]. Treatment effects were observed at 30min or at 24h. In the DMSO/T3 and DMSO/-T3 groups we can observe cells with different morphologies at 30min and 24h, indicating different stages of development. In FORSC/T3, at 30min we observed that the cells' processes were longer and thinner with shrinkage of membrane velum, which persisted until 24h. In FORSC/-T3, at 30min and 24h, the processes were thinner, at 24h they were longer as well, indicating an effect of AMPc in processes elongation. In both SQ groups, cells were polarized and clustered. H-89/T3 groups presented elongated cell bodies and shorten processes at 30min, however, the processes were bigger than control cells at 24h. In H-89/-T3 the membrane velum were larger than DMSO groups. No alterations were observed at 24h. RhoA distribution presented a punctate pattern of distribution both in cell bodies and processes in DMSO/T3 cultured cells. The punctate pattern of distribution was also observed in T3 cultured cells, at both times of drugs treatment. Furthermore, FORSC and H-89 treatment leads to RhoA accumulation in cell bodies. DMSO/-T3 and H-89/-T3 groups also showed an accumulation of RhoA in cell bodies at 30min. In H-89/-T3 and SQ/-T3 groups the protein has both punctate and filamentary pattern. These results showed that the AMPc/PKA pathway and T3 may act together in RhoA distribution with consequences in the elongation of oligodendroglial processes.

T03-02B

Phospholipases A2 isolated from *Micrurus lemniscatus* snake venom inhibits cell proliferation through the activation of p53 in cultured astrocytes

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Endogenous phospholipases A2 have a fundamental role in inflammation, neurodegenerative diseases, apoptosis and cellular senescence. Neurotoxins with PLA2 activity (β-neurotoxins) are found in snake venoms from Elapidae and Viperidae families and these induce neuronal death. Neuronal cultures have been used as a model in order to characterize the mechanisms of action of these

neurotoxins. However, the use of glial cells as a model is scarce. Glial cells, in particular astrocytes, are proliferative cells that participate in homeostasis and defense responses against pathological events in the central nervous system. Moreover, astrocytes are partners at the synapses, composing the tripartite synapse, where pre-synaptic neurons release neurotransmitters that change pos-synaptic neurons and astrocytes activities. Astrocytes in its turn release gliotransmitters that modulate the synaptic transmission by acting on pre- and post-synaptic neurons. The mechanisms of PLA2 neurotoxicity are still in debate and they could be attributed to the enzymatic activity or to the interaction with its own receptor and cellular internalization. The aim of this study was to characterize the effects of two PLA2 (Mlx-8 and Mlx-9) isolated from *Micrurus lemniscatus* venom on cell viability, cell proliferation, cell cycle phases and the intracellular pathways involved, in cultured astrocytes. Astrocytes were obtained by trypsin (0,25%) digestion (10min at 37°C) of eight pineal glands isolated from adult male Wistar rats. The cells were cultivated in 6-well plates (1,2 mL/well; 5 x 10^5 cells/mL) in DMEM medium with 10% fetal calf serum and 1% penicillin-streptomycin (37ºC, 5%CO_2). The cells were incubated with Mlx-8 or Mlx-9 toxins (10 and 100ng/mL) for 2, 6 or 24h. Cell proliferation, cell cycle phases and cell viability were analyzed by flow citometry and MTT assay. P53, p21 and p27 were evaluated by flow cytometry. Cell viability was reduced when astrocytes were incubated with the toxins Mlx-8 and Mlx-9. It was observed that both toxins caused a reduction in cell proliferation and induced cell cycle arrest in G0/G1 or G2/M phases. P53, p21 and p27 were activated by the toxins. These findings suggest that the toxins Mlx-8 and Mlx-9 from *Micrurus lemniscatus* snake venom induce senescence in astrocytes that is mediated by p53, p21 and p27.

T03-03B

The role of CPI-17 in Merlin-dependent small GTPase regulation in oligodendrocytes

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Small GTPases are molecular switches that cycle between inactive (GDP-bound) and active (GTP-bound) conformations. These molecules are involved in complex signalling networks that control cell proliferation, motility, survival and differentiation. Among the major determinants of small GTPase activity are guanine nucleotide exchange factors (GEFs) and GTPase activating proteins (GAPs). In addition, we have previously identified another level of regulation, which depends on the phosphorylation status of the tumour suppressor Merlin. Specifically, phosphorylation of Serine 518 inhibits Merlin activity leading to increased activity of the small GTPase Ras, whereas Merlin activity is restored by the Myosin phosphatase holoenzyme (MYPT1-PP1δ) following dephosphorylation of this residue. MYPT1-PP1δ is inhibited by protein kinase C-potentiated inhibitor protein of 17 kDa (CPI-17) thereby leading to higher concentrations of phosphorylated Merlin and higher Ras activity in cells. So far the regulation of small GTPases by the CPI-17/MYPT1-PP1δ/Merlin axis has only been studied in cell lines. Notably, the myelinating cells in the central nervous system, oligodendrocytes, express CPI-17. Myelin formation and maintenance is dependent on tight control of small GTPase activity. Hence, we sought to analyse the effect of CPI-17 on the myelination process in general and on myelin integrity in particular by using CPI-17 knock-out and overexpression mouse models.

T03-04B

Astrocytic endfeet show unique Ca^{2+} response to osmotic stress

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Astrocytes are crucial for maintaining brain ion and volume homeostasis, in part by Ca^{2+} dependent mechanisms. Here we used the ultrasensitive genetically encoded Ca^{2+} indicator GCaMP6f to study Ca^{2+} signaling in cortical astrocytes in response to hypo-osmotic stress. GCaMP6f was cloned into a recombinant adeno-associated virus (rAAV) vector and driven by the glial fibrillary acidic protein (*Gfap*) promoter. Acute cortical slices from rAAV-*Gfap*-GCaMP6f-transduced animals were subjected to

artificial cerebrospinal fluid (aCSF) with 20% reduction in osmolality, and GCaMP6f fluorescence was imaged by two-photon microscopy. Frequency, duration and amplitude of the astrocytic Ca^{2+} transients were measured before and during hypo-osmotic stress in astrocytic somata, fine processes, perivascular endfeet and subpial endfeet. Hypo-osmotic stress increased the frequency, amplitude and duration of astrocytic Ca^{2+} signals much more in subpial and perivascular endfeet than in other astrocytic compartments. Preliminary analysis reveals that hypo-osmotically evoked Ca^{2+} signals in endfeet were partly sensitive to deletion of dystrophin, an aquaporin-4 anchoring molecule in endfoot membranes, and application of the TRPV4 antagonist HC 067047. We conclude that astrocytic endfeet show a unique hypo-osmotic Ca^{2+} response that depend on dystrophin and TRPV4.

T03-05B

Astrocyte calcium microdomains in response to sensory stimulation *in vivo*

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Question: Intracellular calcium signalling plays an important role in many facets of cellular life. In astrocytes, calcium signalling has been shown to be involved in modulation of neuronal excitability, blood hemodynamic changes and energy metabolism. The complexity and diversity of observable astrocytic calcium signals *in vivo* likely represent different underlying processes, however, the mechanisms involved still need to be resolved.

Methods: By targeted injection of adeno-associated viral vectors, we expressed the genetically encoded calcium indicator GCaMP6s under control of the GFAP promoter specifically in astrocytes of the somatosensory cortex. With two-photon laser scanning microscopy through a chronic cranial window, we recorded cytoplasmic calcium transients. We used a custom, fully automated MATLAB-based algorithm to process images and identify regions of interest (ROIs) based on activity, to detect peaks within those ROIs and classify them based on shape.

Results: We found three distinguishable peak types during spontaneous activity: plateaus, single and multi peak signals. Upon hind paw stimulation, the frequency and area under the curve of all signal types significantly increased in the majority of ROIs compared to spontaneous activity in the same regions. Single whisker deflection led to a significant increase in signal frequency in a subset of ROIs, while peak amplitude and area under the curve decreased in the same regions.

Conclusions: These astrocytic calcium signals may coincide with known neuronal stimulation-evoked responses, as hind paw stimulation triggers an extensive neuronal response while single whisker deflection only induces a sparse activation. Future experiments will include membrane-targeted calcium sensors to better resolve astrocytic microdomains as well as neuronal sensors to combine with astrocytic sensors. This simultaneous imaging approach will help to correlate neuronal and astrocytic calcium signals. The capability of our fully-automated analysis will be expanded to allow longitudinal imaging of the same domains over subsequent days.

T03-06B

c-Jun is activated by LDL receptor-related protein-1 (LRP1) in Schwann cells

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In PNS injury, Schwann cells (SCs) serve as “first responders”, de-differentiating, proliferating, migrating, secreting growth factors and provisional extracellular matrix proteins, and participating in the clearance of degraded myelin. These processes are essential for successful nerve repair. The marked changes in SC phenotype that accompany PNS injury are referred to as “Activation of the SC Repair Program”. LRP1 is a member of the LDL receptor gene family, which includes receptors with endocytic and cell-signaling activity. We have shown that LRP1 is up-regulated in SCs after PNS injury and controls many phenotypic changes associated with activation of the Repair Program. Binding of

ligands to LRP1 in SCs regulates ERK1/2, PI3K, Rac1, RhoA and the unfolded protein response. We have now shown that LRP1 ligands activate the transcription factor c-Jun, which plays an essential role in the SC Repair Program. SCs that were treated with the LRP1 ligands, tissue-type plasminogen activator (tPA) or activated α_2-macroglobulin, demonstrated a substantial increase in phospho-c-Jun. This change was apparent within 5 minutes. c-Jun phosphorylation was blocked by the LRP1 antagonist, RAP, supporting the role for LRP1. The effects of LRP1 on c-Jun suggested that LRP1 may be involved in activation of the SC Repair Program. As a first approach to test this hypothesis, we studied nerve repair following sciatic nerve crush injury in mice in which LRP1 is deleted conditionally in SCs (scLRP1$^{-/-}$ mice). These mice exhibit sustained neuropathic pain after injury (Orita et al., 2013). Ultrastructural analysis of sciatic nerves 23 days after nerve injury revealed abnormal regeneration of Remak bundles. These studies identify a possible link between LRP1 deficiency, abnormal c-Jun activation, altered sensory regeneration, and neuropathic pain. Schwann cell LRP1 emerges as a key regulator of the response to PNS injury.

T03-07B

IL-6 family cytokines selectively activate different signaling pathways in sensory-neuron associated glia and modulate each other signaling in a time and concentration specific manner

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Cytokines from the IL-6 family are central for a variety of neurophysiological processes. This involves cell survival, pain modulation, nerve regeneration and control of the inflammatory response. Even though transmitted by one common receptor subunit, GP130, a number of intracellular signaling pathways are activated in a ligand specific manner. Which ligand activates which pathways with which kinetics, and to what extend do these cytokines and pathways influence each other has not been studied in detail in situ in co-cultures of primary sensory neurons and related glia.

In the present work we have studied on a single cell basis via quantitative automatic “high content screening (HCS)” microscopy the signaling profile of the IL-6 family members Oncostatin M (OSM), LIF and CNTF in glial cells co-cultured with sensory neurons from adult male rat dorsal root ganglia. Focusing on the cytokine-initiated activation/phosphorylation of ERK and STAT3 (phosphorylated at amino residues 705/727), we identified strong differences between the signaling profiles of LIF, CNTF and OSM in S-100+ glia. Despite the differences in signaling, the different members of the IL-6 family influenced each other responses, with LIF and CNTF pretreatment reducing OSM responsiveness in a time, pathway and concentration specific manner. Response length in all conditions was controlled by transcriptional feedback. This was translated in fast but transient responses to the stimulation despite the continuous presence of the stimulus. These differences in signaling were reflected in the expression patterns of early response genes reported to be relevant defining glia functionality in the context of nerve regeneration or pain.

Our results show that distinct members of the IL-6 family have differential effects on glia cells based on their signaling profile. Those effects have the potential to define clinically relevant glia-mediated responses *in vivo*, e.g. during nerve regeneration. Our detailed pathway analysis allows us also to suggest ways for response manipulation.

T03-08B

Calcium regulation of mitochondrial respiration in astrocytes

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Calcium regulation of brain mitochondrial respiration is essential to match energy demand to ATP production. Calcium signaling in mitochondria may regulate respiration through: i) Ca^{2+} entry in the organelle across the calcium uniporter (MCU), activating mitochondrial dehydrogenases and F_0F_1-ATPsynthase; or ii) by activating Ca^{2+}-binding mitochondrial carriers, ARALAR/AGC1 and SCaMCs from the outer face of the inner mitochondrial membrane, which involves metabolite supply. The ARALAR/AGC1 pathway contributes very significantly to workload and calcium-induced respiration in neurons (Llorente-Folch, Rueda et al., 2013). In brain astrocytes ARALAR levels are undetectable and Ca^{2+} regulation of respiration is expected to involve the MCU pathway and SCaMC-3 To explore this possibility, we have studied the metabolic response of astrocytes to different workloads and the possible role of Ca^{2+} in response.

Studies in cultured astrocytes have shown that ATP induces cytosolic Ca^{2+} signals through P2X and P2Y receptors. ATP caused an increase in glucose utilization and lactate production in cultured astrocytes, and also a rise in OCR (Seahorse XF24 Extracellular Flux Analyzer), all of which were dependent on the presence of external Ca^{2+}. Maximal uncoupled respiration was also Ca^{2+}-dependent. The specific role of MCU and SCaMC-3 in this activation remains to be studied.

Astrocytes also have glutamate receptors, mostly metabotropic, and glutamate transporters. L-Glutamate (200-500 μM) increased glucose utilization, lactate production and acutely stimulated oxygen consumption. Glutamate-stimulation of respiration depended on the presence of external Ca^{2+}, but not maximal uncoupled respiration. To test whether this was due to its effects as respiratory substrate rather than signaling molecule, we used D-aspartate, a transportable but non-metabolizable glutamate analog. D-aspartate (500 μM) acutely stimulated respiration in a way similar to L-glutamate but failed to increase maximal uncoupled respiration, clearly showing that glutamate is used as respiratory substrate by cultured astrocytes. Further studies are required to dissect the role of MCU and SCaMC-3 in Ca^{2+}-dependent glutamate-induced respiration.

Llorente-Folch, I., Rueda, C. B., Amigo, I., del Arco, A., Saheki, T., Pardo, B., & Satrustegui, J. (2013). Calcium-regulation of mitochondrial respiration maintains ATP homeostasis and requires ARALAR/AGC1-malate aspartate shuttle in intact cortical neurons. *J Neurosci, 33*(35), 13957-13971.

T03-09B

Cross-talk of signaling and energy-delivering processes in astrocytes: interaction of carnitine transporter OCTN2 with phosphatase PP2A

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Question: L-carnitine is essential for translocation of acyl moieties for the mitochondrial β-oxidation of fatty acids, a process which in the brain takes place in astrocytes. OCTN2 (SLC22A5) transporter, present in astrocytes, catalyses carnitine accumulation and its mutations were correlated with several pathologies, including those affecting brain (Reye-like syndrome). Our previous studies showed that OCTN2 increased activity and its presence in plasma membrane of astrocytes have been correlated with the protein kinase C activation, although no phosphorylation of the transporter was detected. Therefore, the present study was focused on identification of OCTN2-interacting partners in astrocytes and finding their role in transporter trafficking to the plasma membrane.

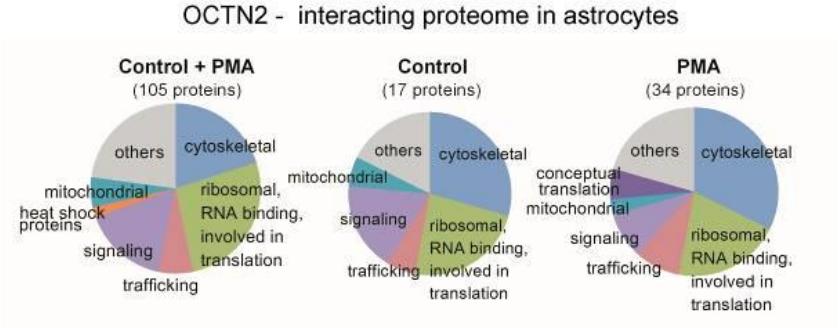
Methods: The mass spectrometry analysis identified several cytoskeletal, ribosomal, mitochondrial, and heat-shock proteins as well as the proteins involved in signaling pathway and trafficking. Proteins from signaling pathway were selected and their interaction with OCTN2 was further studied by immunoprecipitation and immunocytochemistry experiments.

Results: The detailed biochemical analysis showed co-precipitation of OCTN2 with PP2A phosphatase catalytical (C) and structural (A) subunits, as well as with its regulatory subunits - striatin and SG2NA. Since both striatin and SG2NA bind caveolin-1, whose direct interaction with OCTN2 was shown previously, formation of a multiprotein complex has been postulated.

Conclusions: Interaction of OCTN2 with PP2A can arrest OCTN2 in the dephosphorylated state. The observed interaction could affect the brain *in vivo*, especially because carnitine homeostasis is involved in certain neurodegenerative diseases, e.g. autism and Alzheimer's disease.

This work is financed by grant 2012/07/B/NZ3/00225 from National Science Centre in Poland.

Image



T03-10B

An organic device for stimulation and optical read-out of calcium signalling in primary rat cortical astrocytes

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Astroglial ion channels and calcium signalling play a central role in the physiology and pathophysiology of the Central Nervous System. In this context, increasing efforts are needed to generate innovative tools for monitoring astrocytes biochemical or bioelectrical activity in vitro and in vivo.
Organic field effect devices have a great potential for generating advanced biomedical tools to enable real-time recording and manipulation of communication signals between neural cells.

We previously reported on transparent Organic Cell Stimulating and Sensing Transistors (O-CSTs) that provide bidirectional stimulation and recording of primary neurons. The transparency of the device also allows the optical imaging of the modulation of the astroglial calcium signalling bioelectrical activity. Here we explore O-CST functionality to stimulate, evoke and control astroglial calcium signalling and whole cell conductance in primary cultured astrocytes.

We found that primary astroglial cells can adhere, grow and differentiate on the perylene based field-effect transistor. Furthermore does the organic material preserve astrocytes electrophysiological properties. We show, that the O-CST provides stimulation and thereby evokes intracellular astrocytic calcium response, which can be determined by calcium imaging. The evoked signal was blocked by carbenoxolone and Ruthenium red, thus suggesting involvement of Connexins and TRPV channels. By means of patch-clamp analyses, we explore the effect of the stimulation on the whole-cell conductance of patched astrocytes. We found that the stimulation lead to an exclusive increase in the inward current that could be prevented by application of Ruthenium Red prior to stimulation. This finding suggests a contribution of the transient receptor potential (TRP) channels, of which TRPV-4 has been shown in former studies to mediate Ca^{2+} influx in astrocytes. Molecular modelling of field distribution obtained by O-CST is also in agreement with experimental data.

Our organic cell stimulating and sensing device paves the way to a new generation of devices for stimulation, manipulation and recording of astroglial cells' bioelectrical activity *in vitro*. Sponsored by: EU-ITN-OLIMPIA GA 316832

T03-11B

CREB: a new player in the regulation of astrocytic calcium signalling

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Astrocytic regulation of synaptic transmission and formation of new memories relies on gliotransmission and hence calcium signalling. However little is known about the mechanisms that control calcium responses in astrocytes. We studied whether astrocytes present long-term regulation of intracellular calcium increases in cultured rat cortical astrocytes. To do so, CREB-dependent transcription was triggered by 1-hour pulses with noradrenaline (10μM) or ATP (100μM), which we have previously shown to activate CREB-dependent transcription in astrocytes within 6 hours, and cytosolic calcium responses were assessed by calcium imaging in Fluo-4 loaded cells challenged by gliotransmitters (ATP, NA and ET-1). Such endogenous CREB activation reduced calcium responses to agonists by 15-38% and this reduction was reversed by the viral transduction of a dominant negative form of CREB (A-CREB).Likewise, viral transduction of a constitutively active form of CREB (VP16-CREB) caused a 20-30% decrease in gliotransmitter-induced calcium response, as compared to astrocytes infected with an empty virus (Null). We next analysed the calcium signalling pathways shaped by CREB activation. CREB-induced decrease in calcium transients was still observed in the absence of extracellular calcium and in the presence of Ned-19, an inhibitor of calcium release from acidic stores. Preliminary results using the new calcium probe G-CEPIA1er showed small differences of calcium release from the endoplasmatic reticulum between VP16-CREB and Null-infected astrocytes. Finally, ATP-induced mitochondrial calcium rises, monitored with Rhod-2, were around 3 times higher in VP16-CREB overexpressing than in Null cells. We thus conclude that CREB changes calcium excitability in astrocytes mainly by increasing calcium buffering by mitochondria.

T03-12B

A functional metabotropic-like NMDAR in rat cultured astrocytes

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The ionotropic glutamate N-methyl D-aspartate (NMDA) receptor (NMDAR) is critical for CNS functions, but its expression and function in astrocytes is still a matter of research and debate. Here, we demonstrate immunofluorescence (IF) labeling in rat cultured cortical astrocytes (rCCA) of all NMDAR subunits, with phenotypes suggesting their intracellular transport, and their mRNA were detected by qRT-PCR. IF and Western Blot revealed GluN1 full length synthesis, subunit critical for NMDAR assembly and transport, and its plasma membrane localization. Functionally, we found an iCa^{2+} rise after NMDA treatment in Fluo-4-AM labeled rCCA, an effect blocked by the NMDAR competitive inhibitors D(-)-2-amino-5-phosphonopentanoic acid (APV) and Kynurenic acid (KYNA), and dependent upon GluN1 expression as evidenced by siRNA knock down. Surprisingly, the iCa^{2+} rise was not blocked by MK-801, an NMDAR channel blocker, or by extracellular Ca^{2+} depletion, indicating flux-independent NMDAR function. In contrast, the IP_3 receptor (IP_3R) inhibitor XestosponginC did block this response, whereas a Ryanodine Receptor inhibitor did so only partially. Furthermore, tyrosine kinase inhibition with genistein enhanced the NMDA elicited iCa^{2+} rise to levels comparable to those reached by the gliotransmitter ATP, but with different population dynamics. Finally, NMDA depleted the rCCA mitochondrial membrane potential (mDy) measured with JC-1. Our results demonstrate that rCCA express NMDAR subunits which assemble into functional receptors that mediate a metabotropic-like, non-canonical, flux-independent iCa^{2+} increase.

T03-13B

Differential secretion of peptidergic vesicles in astrocytes and neurons

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Peptidergic secretion from neural cells can be either constitutive or regulated. In the constitutive secretory pathway release, vesicles do not accumulate to an appreciable degree and fuse in a stimulus-independent manner with the plasma membrane. On the other hand, in the regulated secretion newly synthesized products are stored in specialized vesicles/granules and accumulated within the cell. Upon stimulation, the contents of these vesicles undergo rapid release from the cell. Originally, neurons were considered the unique neural cell type displaying a regulated peptide secretion pathway. Recently, is has been shown that astrocytes are also competent to release peptidergic messengers in a regulated fashion. Here we studied two hallmark proteins of the regulated secretory pathway, Secretogranin III (SgIII) and Carboxypeptidase E (CPE), in astrocytes and neurons. Both proteins are abundantly expressed by glial and neuronal cells in vivo and in vitro. In acute ex-vivo cortical preparations (brain slices), a stimulus-independent release were observed for both the unprocessed and mature forms of SgIII and CPE. Using different stimuli and blocking agents release of matured forms of SgIII and CPE were differentially altered. To analyze secretion dynamics of peptidergic granules in astrocyte and neuron populations we used cell type-enriched primary cultures. A differential expression of granule processing enzymes and specific processed forms of SgIII and CPE was observed in astrocytes and neurons. In absence of stimuli, newly produced SgIII and CPE were rapidly secreted from astrocytes, whereas basal secretion was extremely low in neurons. Conversely, calcium-triggered secretion was moderate in astrocytes but high in neurons. These results show that neurons and astrocytes display similar molecular components of the regulated secretory pathway, but differential dynamics of the release. Moreover, an astroglial regulated secretory pathway of peptidic messengers in vivo is also discussed.

T03-14B

Src-like tyrosine kinases mediate amyloid ß-induced myelin dysregulation in Alzheimer´s disease models

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A reduction in the size of *corpus callosum* as a consequence of myelin loss and oligodendrocyte cell death has been reported to occur in Alzheimer´s disease (AD) and in transgenic mice models. These alterations may slow action potential propagation and contribute to AD progression. Here, we have characterized the effects of oligomeric amyloid ß peptide (Aß) in primary oligodendrocyte and cerebellar organotypic cultures. First, we observed that Aß promoted the transition of early oligodendrocyte progenitors to late progenitor stages, and oligodendrocyte maturation. In addition, Aß peptide induced myelin basic protein (MBP) expression and myelination. To further investigate the mechanisms underlying Aß-mediated myelin changes, we analyzed the activation of Src family protein kinase and CREB, two key signalling pathways involved in myelin synthesis. We found that Aß caused a sustained Src and CREB protein phosphorylation in primary cultures of oligodendrocytes, and specific pharmacological inhibition of both pathways reduced Aß-induced MBP upregulation. Moreover, Aß enhanced the expression of other major components of CNS myelin including PLP and CNPase, and favored remyelination after lysolecithin-induced demyelination in cultured cerebellar slices. Importantly, Aß-mediated MBP upregulation in cultured slices was blocked by PP2, a Src family kinase inhibitor that potently inhibits Lck/Fyn proteins which suggests that they play a key role in that process. We next examined whether Aß accumulation *in vivo* was associated with MBP dysregulation. Western blotting of MBP and Aß oligomers in *hippocampus* and *corpus callosum* of 18-month old

triple-transgenic mouse model of AD showed an increase of MBP levels in both regions that correlated with Aß oligomer burden. Consistent with those findings, MBP levels in frontal cortex of AD brains were significantly higher than in age-matched non-demented controls. Together, these data support a role of Aß and Src-like kinases in the pathophysiology of myelin in AD though the sequence of events leading to upregulation of MBP levels in this disease remains to be clarified.

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T03-15B

Astrocyte shape changes and tonic cAMP signalling

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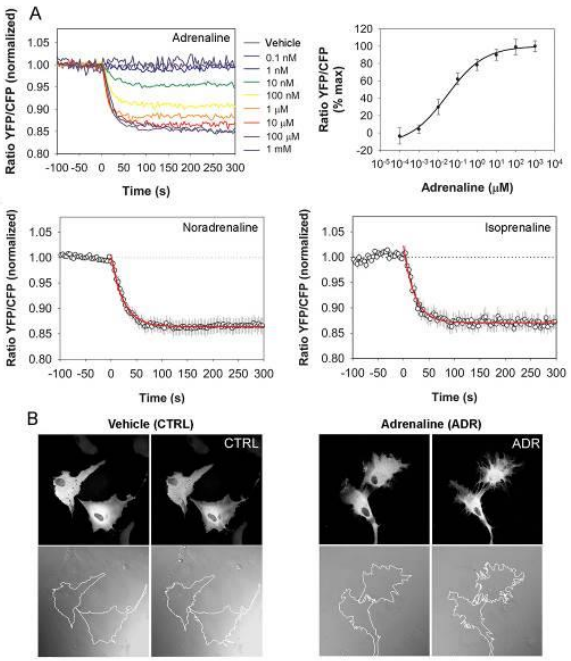
The morphology of astrocytes, likely regulated by cAMP, determines the structural association between astrocytes and the synapse, consequently modulating synaptic function in health and disease.β-Adrenergic receptors (β-AR), which increase cytosolic cAMP concentration ($[cAMP]_i$), may affect cell morphology. However, the real-time dynamics of β-AR-mediated cAMP signalling in single live astrocytes and its effect on cell morphology are sparse. We used the fluorescence resonance energy transfer (FRET)-based cAMP biosensor Epac1-camps to study time-dependent changes in $[cAMP]_i$ while morphological changes in primary rat astrocytes were monitored by real-time confocal microscopy (Vardjan et al., 2014). Stimulation of β-AR by adrenaline, noradrenaline and isoprenaline, a specific agonist of β-AR, rapidly increased $[cAMP]_i$ with time-constants of ~15 s (Figure 1A). The FRET signal response, mediated via β-AR, was faster than in the presence of forskolin (2-fold) and dibutyryl-cAMP (>35-fold), which directly activate adenylyl cyclase and Epac1-camps, respectively, likely due to slow entry of these agents into the cytosol. Oscillations in $[cAMP]_i$ have not been recorded in astrocytes, indicating that cAMP-dependent processes modulate cellular processes tonically. Most Epac1-camps expressing astrocytes revealed a morphological change upon β-AR activation and attained a stellate morphology within 1 h (Figure 1B). The morphological changes exhibited a bell-shaped dependency on $[cAMP]_i$, indicating that maximal morphological changes are limited to an optimal (narrow) range of $[cAMP]_i$. The rapid 5-10% decrease in cell cross-sectional area and the 30-50% increase in cell perimeter are likely due to withdrawal of the cytoplasm to the perinuclear region and the appearance of protrusions on the surface of astrocytes (Table 1). Because astrocyte processes ensheath neurons, β-AR/cAMP mediated morphological restructuring can modify the geometry of the extracellular space, affecting synaptic, neuronal and astrocyte functions in normal and pathological conditions.

Ref.: Vardjan N, Kreft M and Zorec R. (2014) Dynamics of β-Adrenergic/cAMP signaling and morphological changes in cultured astrocytes. Glia. 62(4): 566-579.

Image

Figure 1



Table

Table 1: Morphometric analysis of astrocyte shapes before and after stimulation of β-adrenergic/cAMP-signalling pathway

Type of Stimulus	CSF Before stimulation	CSF After stimulation	Decrease in CSF (%)	*n*
Vehicle	0.218 ± 0.020	0.198 ± 0.023	10.8 ± 5.5	15
Adrenaline (1 μM)	0.193 ± 0.026	0.094 ± 0.017	51.0 ± 5.4**	9
Noradrenaline (1 μM)	0.244 ± 0.028	0.142 ± 0.023	38.3 ± 7.7**	12
Isoprenaline (1 μM)	0.195 ± 0.032	0.119 ± 0.030	43.3 ± 6.5*	6

Cell Shape Factor (CSF) was calculated according to the equation: $4\pi A/P^2$, where A is the cell cross-section area and P the cell perimeter. This measure ranges from 0 to 1 between extremes of a line and a perfect circle (one-way ANOVA compared to vehicle. $^{**}P < 0.001$, $^{*}P < 0.05$). *n* denotes the number of experiments.

T03-16B

Schwann cell autophagy, myelinophagy, initiates myelin clearance from injured nerves

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Although Schwann cell myelin breakdown is a universal outcome of a remarkably wide range of conditions that cause disease or injury to peripheral nerves, the cellular and molecular mechanisms that make Schwann cell-mediated myelin digestion possible, have not been established. We report that Schwann cells degrade myelin after injury by a novel form of selective autophagy, myelinophagy.

Autophagy is up-regulated by myelinating Schwann cells after nerve injury, myelin debris is present in autophagosomes, and pharmacological and genetic inhibition of autophagy impairs myelin clearance. Myelinophagy is positively regulated by the Schwann cell JNK/c-Jun pathway, a central regulator of the Schwann cell reprogramming in injured nerves. We also present evidence that myelinophagy is defective in the injured CNS. These results reveal an important role for inductive autophagy during Wallerian degeneration, and point to a potential mechanistic target for accelerating myelin clearance and improving demyelinating disease.

T03-17B

A crosstalk between rock and NADPH-oxidase mediates the microglial inflammatory response

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Introduction: Brain renin-angiotensin system (RAS) plays a major role, via angiotensin type 1 (AT1) receptors, in the regulation of neuroinflammation, oxidative stress and progression of dopaminergic (DA) degeneration. Angiotensin (AII)-induced activation of the microglial NADPH-oxidase complex and microglial Rho-kinase (ROCK) are particularly important in this respect. In the present study, we investigated the possible existence of a crosstalk between ROCK and NADPH-oxidase that leads to microglial activation.

Methods: To study the interaction between the AII-induced NADPH-oxidase and ROCK activities, young adult rats were treated with AII**,** AII plus ROCK inhibitor Fasudil; or AII and the NADPH-oxidase inhibitor apocynin. 24h after receiving the AII injection, animals were killed and nigral region processed for western blot (WB) and ROCK and NADPH-oxidase activities.

Cultures of the murine N9 microglial cell line and primary microglia were exposed to AII alone or to AII plus a second ROCK inhibitor, Y-27632, or AII and apocynin. To study the involvement of AII derived superoxide in ROCK activation, cultures were pretreated with antioxidant N-acetyl-cysteine. To study the involvement of NFkb in the AII-induced increase in ROCK activity, cultures were treated with the NFkb inhibitor PDTC. To investigate the involvement of p38 mitogen-activated protein kinase (p38-MAPK) in the AII-induced increase in NADPH activity, cultures were treated with the p38-MAPK inhibitor SB-203580. Cells were finally processed for WB and analysis of ROCK and NADPH-oxidase activities.

Results: We found that, in the substantia nigra of rats, NADPH-oxidase activation was involved in AII-induced ROCK activation, which, in turn, was involved in AII-induced NADPH-oxidase activation. In N9 microglial cell line and primary microglial cultures, a crosstalk signaling between NADPH-oxidase and ROCK occurred in a positive feedback fashion during AII-induced microglial activation. AII-induced NADPH-oxidase activation and superoxide generation led to NFkb translocation and ROCK activation. ROCK activation was involved in regulation of NADPH-oxidase activation via p38-MAPK. Moreover, ROCK activation, via NFkb, upregulated AT1 receptor expression in microglial cells through a feed-forward mechanism.

Conclusions: The present results show that NADPH-oxidase and ROCK, pathways known to be responsible for major components of the microglial response, are linked by a common mechanism that may constitute a basic means of coordinating the microglial response.

Poster topic 04
Cytoskeleton

T04-01A

JMY, an actin-nucleator involved in oligodendrocyte process extension and early axon-glia interaction

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The formation of myelin sheath around axons in the central nervous system is carried out by oligodendrocytes (OL). The ensheathment and wrapping steps of myelination are extremely complex and highly dynamic events that require a rearrangement of the cytoskeleton. Although much remains to be learned about this process, it is clear that actin-related proteins are important players and regulators of these events. We are interested in further characterizing the actin-regulating protein network that dictates how OLs extend processes, interact with the axon and starts wrapping around it. We found that a novel protein, JMY, also known as junction-mediating and regulatory protein p53 cofactor, is upregulated in the processes of OLs, when compared with its expression in the soma, during the initial phase of process extension. JMY has been described as a dual functional protein that can act as an actin nucleator when in the cytoplasm, through Arp2/3 activation or trough direct actin nucleation by itself, and can also be a p53 cofactor when in the nucleus. Therefore, we asked how JMY is modulating process extension and early axon-glia interaction and if it has a function in axon ensheathment and wrapping. Using primary cultures of rat oligodendrocyte progenitor cells, we observed that JMY is present during OL differentiation *in vitro*, with maximal expression in fully differentiated, mature OLs. Knockingdown JMY in OLs *in vitro* leads to dramatic morphological defects, including fewer and shorter processes with reduced branching. However, depletion of Jmy does not seem to impact the expression of myelin genes, such as MBP (myelin basic protein). This presents as a very interesting example to dissect how ubiquitous molecules differentially regulate form and function, especially in a cell where the two are intrinsically linked. We are now using a co-culture subsystem of dorsal root ganglion (DRGs) and oligodendrocytes lacking JMY to assess whether JMY is required for normal axon-glia early interaction. In the future, we plan to perform live cell imaging of these co-cultures to get insight on how this dynamic process takes place and the OL cell shape impacts the formation of the myelin sheath around an axon.

T04-02A

Dystonin loss-of-function in oligodendrocytes does not impair migration, differentiation, or myelination

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Dystonin (also known as BPAG1) is a large cytoskeletal linker protein with both actin- and microtubule-binding domains. Dystonin loss-of-function in humans results in a sensory neuropathy called Hereditary Sensory and Autonomic Neuropathy type VI (HSAN-VI). In mice, mutations in the gene encoding dystonin lead to *dystonia musculorum* (*dt*). The disease presents with severe joint contractures and a multitude of dysautonomias, which is ultimately fatal. While dystonin loss-of-function primarily leads to sensory neuron degeneration, peripheral myelination is also compromised due to Schwann cell differentiation abnormalities. Interestingly, central nervous system (CNS) neurons appear to be little affected by loss of dystonin, suggesting that they possess a compensatory mechanism not present in sensory neurons. We sought to determine if this dichotomy also exists between Schwann cells and oligodendrocytes (OLs), the CNS myelinating cells. To address this,

primary OL cultures were established from a severe *dt* model (dt^{27J}), and assessed for differentiation capacity as well as maturation marker expression by immunofluorescence. There was no deficiency in branching ability, or in the expression of myelin markers myelin-associated glycoprotein (MAG) or myelin basic protein (MBP), in dt^{27J} OLs relative to their wild type littermates. Using a recently developed oligosphere assay, we further analysed the ability of primary oligodendrocyte progenitor cells (OPCs) to migrate and again found no difference between dt^{27J} and wild type. Finally, *in vivo* analysis of OL myelination was done by electron microscopy on phenotype-stage optic nerve. While some myelin abnormalities were observed in dt^{27J} optic nerve, G-ratio and percent myelinated axon analyses revealed no significant differences when compared to wild type. These data suggest that, like CNS neurons, OLs also possess a compensatory mechanism for overcoming the loss of dystonin, setting them apart from their Schwann cell counterparts in the periphery.

T04-01B

Astroglial architecture of Squamata as compared to the astroglia of Crocodilia and Testudines. A GFAP study

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The Crocodilia belongs to Archosauria the main group of Diapsida which contains also birds, dinosaurs and pterosaurs. The Squamata (snakes and lizards) are positioned in another diapsid reptilian branch, Lepidosauria. Testudines were held formerly a part of the most ancient group, Anapsida, but their position has been challenged and recently they are also classified as diapsid reptiles. Mammals represent the crown group of Synapsida a clad diverged early from the other Amniotes. It was found that there are similar features of astrglia of mammals and birds which developed during parallel, separate evolutions. Beside the predominancy of astrocytes the most important phenomenon is the appearance of large brain areas free of GFAP or at least very poor in it but capable of GFAP expression following lesions (Kálmán, 2002). These areas were usually the most advanced, most expanded and most plastic areas of either mammal or bird brains. No similar areas and no predominancy of astrocytes were observed in either turtles or crocodilians (Kálmán et al., 1994, 2001). Present study searches for these phenomena of astroglial evolution in the lepidosaur lizards and snakes.

Six species were studied, lizards: *Pogona witticeps* (Agamidae), *Eublepharis macularis* (Gekkonidae), and *Chameleo calypratus* (Chameleonidae), and snakes: *Epicrates cenchria maura* (Boidae), *Python regius* (Pythonidae), *Elaphe guttata* (Colubridae). The animals were obtained from breeders. They were sublethally overanasthetised with Nembutal and transcardially perfused with paraformaldehyde solution (4% in phosphate buffer-saline). Following two days postfixation, the brains were embedded into agarose and series of coronal sections (50-70 µm) were cut by a Vibratome, and floating sections were processed according to the immunoperoxidase protocoll. As a primary antibody monoclonal moues anti-GFAP (Novocastra) were used in a dilution of 1:100.

The immunostaining revealed that the main astroglial type is the radial ependymoglia. Non-radial long fibers penetrated this system around the large vessels. Astrocyte-like elements were frequent in several areas, e.g. in the pallium and the striatum but nowhere predominated. Astroglia-poor areas were found in the dorsal pallium, in the septum, in the dorsal ventricular ridge and in the hypothalamus. They were especially extended in the *Python regius.*

The evolutionary and functional importance of these areas have not been understood yet. Similar areas were not seen in crocodilians and turtles (Kálmán et al., 1994, 2001).

T04-02B

Stress in mice rapidly changes enteric glial morphology through cytoskeletal reorganization

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Question: Stress activates physiological responses through activation of the sympathetic nervous system that allows the body to cope with the challenges of a given environment. It is increasingly evident that stress can affect biological functions in various organs in the periphery as well as in the brain. However, stress has also been associated with gut dysfunction and exacerbation of inflammation in irritable bowel syndrome and inflammatory bowel disease, respectively. Little is known about the mechanisms by which stress causes these effects in the gastrointestinal tract. We recently showed that enteric glia in the myenteric plexus are targets of sympathetic innervation and therefore we investigated how stress affects enteric glia using mouse stress models. In particular, we focused on the early responses within 15 minutes of stress in enteric glia in the myenteric plexus of the mouse distal colon.

Methods: Mice were exposed to acute forced swim for 15 min (n=3) or chronic forced-swim stress for 15min/day for 5 consecutive days (n=4) as well as a novel stress (n=5) where mice were introduced to a predator odor after 4 days of chronic forced-swim stress or no stress controls (n=3). GFAP expression from 10 ganglia in the myenteric plexus of each animal was used to assess changes in enteric glial morphology.

Results: We found that there is a significant increase in the number of enteric glia that showed a high GFAP immunoreactivity along with formation of thick GFAP immunoreactive processes in the novel stress mice compared to the chronic stress mice, acute stress mice and no stress control mice (Figure 1). The difference was most pronounced between the novel stress mice compared to the chronic stress mice, which is in line with the paradigm that a novel stress generates an exaggerated response whereas a chronic stress can lead to a suppressed response. The changes in the glial morphology revealed by GFAP immunoreactivity are reminiscent of reactive gliosis, but the rapid nature of the change suggests that it is caused by transient cytoskeletal reorganization rather than the increase in the GFAP gene expression.

Conclusions: These results show that stress can induce significant changes in the enteric glia that may disturb the normal physiological function of the gut and shed light on the pathogenesis of stress induced intestinal disorders.

Image

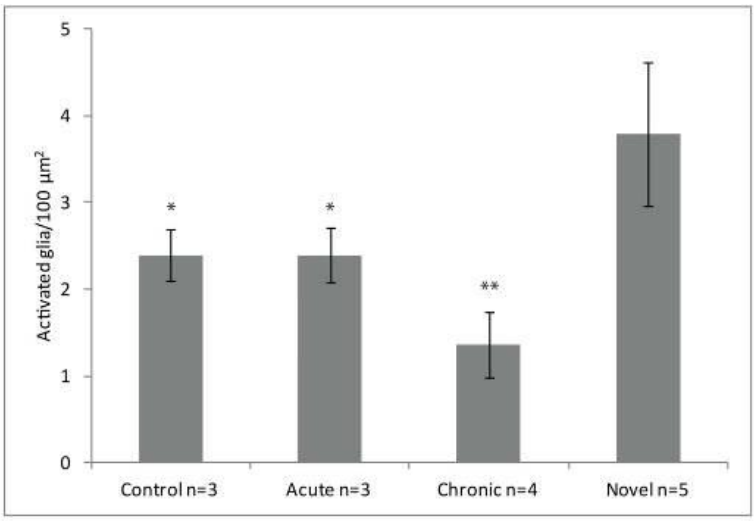

Figure 1. Novel stress induced changes in enteric glial morphology. Activated enteric glia that showed a high GFAP immunoreactivity along with formation of thick processes were counted from 10 randomly selected ganglia from each animal and expressed by the activated glia in 100 square microns. (* $p<0.05$, ** $p<0.0001$, compared to Novel)

Poster topic 05
Degenerative disease, toxicity and neuroprotection

T05-01A

Microglial phagocytosis-apoptosis coupling: a widespread response disturbed in epilepsy

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Phagocytosis is a highly conserved process essential to maintain tissue homeostasis. However, little is known about its dynamics in the adult brain. Using as a model the adult neurogenic cascade, where the majority of the newborn cells undergo apoptosis, we have defined a series of parameters that establish the baseline efficiency of microglial phagocytosis in the adult brain. In physiological conditions, apoptotic cells are rapidly and efficiently phagocytosed by microglia. When subjected to different phagocytic challenges as inflammation *in vivo* (systemic LPS), chronic inflammation *in vivo* (Omega 3 defficient diet), or excitotoxicity *in vitro* (NMDA in organotypic slices), microglia stand up to the increased apoptosis by raising proportionally their phagocytic capacity - hence, phagocytosis remains coupled to apoptosis. In contrast, in an *in vivo* model of epilepsy induced by kainate administration, a major neurological disorder characterized by excitotoxicity and inflammation, microglial phagocytosis is strongly uncoupled from apoptosis. These new parameters we introduce enable us to describe microglial phagocytosis dynamics in both health and disease and represent a very powerful tool to detect disruptions in this physiological process. The consequences and mechanisms underlying this phagocytosis-apoptosis uncoupling will be discussed.

T05-02A

Microglial phagocytosis is impaired in chronic mouse and human MTLE and correlates with inflammation

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In physiological conditions in the adult hippocampus, apoptotic cells are rapidly and efficiently phagocytosed by microglia. We have observed that during aging, inflammation, and excitotoxicity, microglia responded to the increase in apoptosis by adjusting proportionally their phagocytosis. Conversely, in a mouse model of mesial temporal lobe epilepsy (MTLE) by intrahippocampal administration of kainic acid (KA), microglial phagocytosis was reduced as early as 6 hours after injury, and continued to be impaired in the long term. Importantly, this phagocytic blockade led to the accumulation of non-phagocytosed apoptotic cells, and contributed to the development of an inflammatory response. Unexpectedly, in the subacute phase (3-7 days) of MTLE, microglia showed a hypertrophic, seemingly amoeboid morphology that was related to the cells becoming multinucleated. Further, we also detected some cases of phagoptosis or engulfment or non-apoptotic cells. In later stages (4 months) of MTLE, microglial phagocytosis remained impaired. Importantly, the microglial phagocytosis impairment was observed in human hippocampal tissue from MTLE patients. In the human tissue, we found the same kind of phagocytosis observed in the mouse brain by terminal or en passant branches of microglia. In addition, we observed a unique type of phagocytosis in which several microglial nuclei formed a surrounding the apoptotic cell, in an aster-like structure. These results demonstrate that the impairment of microglial phagocytosis is a novel mechanism contributing to the pathophysiology of epilepsy.

T05-03A

Neuronal hyperactivity uncouples microglial phagocytosis and leads to delayed self-clearance and inflammation

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In physiological conditions, aging, inflammation and excitotoxicity microglial phagocytosis is fast and efficiently coupled to apoptosis in the adult hippocampus, but becomes impaired in a mouse model of epilepsy by intrahippocampal injection of kainic acid (KA). Here we studied the possible mechanisms underlying this uncoupling. To test whether the phagocytic blockade induced by seizures was mediated by the direct effect of KA, we first analyzed the expression of glutamate ionotropic and metabotropic receptor subunits in FACS-sorted microglia. Hippocampal microglia expressed a residual mRNA amount of most subunits, which was unlikely to lead to the formation of functional receptors. In addition, KA had a small effect on microglial phagocytosis in primary cultures and no effect in organotypic cultures, suggesting that the effects of seizures in phagocytosis in vivo are not directly mediated by KA on microglia. Next, we studied the extracellular nucleotide ATP, a well-known "find-me" signal released by apoptotic cells as well as during seizures. We were able to mimic the uncoupling observed in vivo by disrupting ATP gradients in organotypic slices, suggesting that neuronal hyperactivity interferes with "find-me" signals via ATP. Ultimately, microglial phagocytosis impairment leads to the accumulation of non-phagocytosed apoptotic cells and correlates with the development of an inflammatory response in vivo. These results suggest that the impairment of microglial phagocytosis contributes to the early pathophysiology of epilepsy and possibly other neurodegenerative and neurological disorders characterized by neuronal death and inflammation.

T05-04A

The synthetic microneurotrophin BNN27 in demyelination: the role of glia in neuroprotection

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Neurotrophins (NGF, BDNF, NT3 and NT4), acting through the Trk and/or the p75NTR receptors, are able to regulate the regenerative capacity of the nervous system, participating in neuronal survival, differentiation and repair. These molecules have neuroprotective and neurogenic effects in various neurodegenerative diseases. Dehydroepiandrosterone (DHEA) is a small endogenous neurosteroid that binds with high affinity to the NGF receptors. DHEA exerts potent neuroprotective effects, inducing the expression of anti-apoptotic Bcl-2 proteins by directly interacting with TrkA and p75NTR receptors. Thus, it efficiently induces TrkA phosphorylation and NGF receptor-mediated pro-survival signaling, resulting in the induction of neuroprotective mir21 and pro-survival kinases. However, DHEA is metabolized to estrogens and androgens, affecting the endocrine system and increasing the risk for hormone-dependent tumors.

BNN27, a 17-spiro analog of DHEA, exhibits strong neuroprotective properties, which are deprived of endocrine effects. We showed previously that BNN27 positively affects the anti-inflammatory response and induces the activation of Treg lymphocytes in Experimental Autoimmune Encephalomyelitis (EAE), a mouse model resembling multiple sclerosis (MS).

Our aim here is to investigate the primary neuroprotective and/or restorative effects of BNN27 in CNS populations. We used the cuprizone method of demyelination, an established rodent model characterized by the degeneration of the oligodendrocytes, where the process of de/re-myelination

can be studied without the direct involvement of the immune system. Using immunohistochemical and morphological approaches we found a significant decrease in the level of demyelination, astrogliosis and microgliosis in the BNN27-treated mice, compared to the controls, accompanied by an increased level of mature oligodendrocytes. Based on these in vivo results our working hypothesis tests the possibility that BNN27, acting through TrkA and p75NTR receptors, is implicated in oligodendrocyte survival, which is one of the main processes under study with the aim of improving therapeutic treatments of patients with MS.

We are currently investigating the effect of BNN27 on the migration potential of oligodendrocyte precursors (OPCs) from the subventricular zone towards the adjacent corpus callosum (one of the primarily demyelinated area in the cuprizone model), and the putative involvement of BNN27 in OPCs survival, proliferation and maturation, using in vitro experiments.

T05-05A

Role of extracellular calcium and mitochondrial oxygen species in psychosine-induced oligodendrocyte cell death

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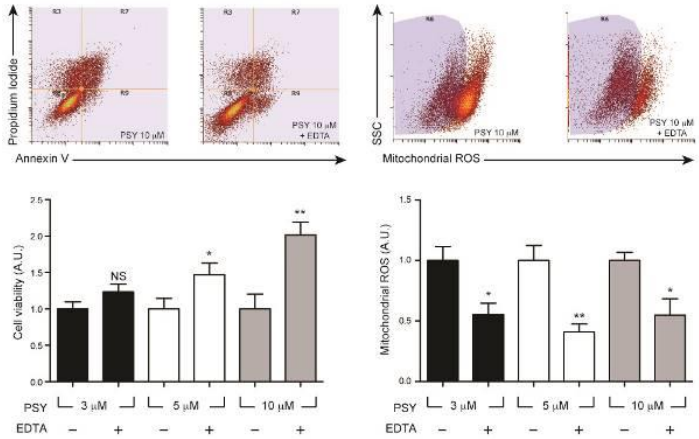
Globoid cell leukodystrophy (GLD) is a metabolic disease caused by mutations in the galactocerebrosidase (GALC) gene. GALC is a lysosomal enzyme whose function is to degrade galacto-lipids, including galactosyl-ceramide and galactosylsphingosine (psychosine, PSY). GALC loss of function causes progressive intracellular accumulation of PSY. It is widely held that PSY is the main trigger for the degeneration of myelinating cells and progressive white-matter loss. However, still little is known about the molecular mechanisms by which PSY imparts toxicity.

Here, we address the role of calcium dynamics during PSY-induced cell death. Using the human oligodendrocyte cell line MO3.13, we report that cell death by PSY is accompanied by robust cytosolic and mitochondrial calcium (Ca2+) elevations, and by mitochondrial reactive oxygen species (ROS) production. Importantly, we demonstrate that the reduction of extracellular calcium content by the chelating agent ethylenediaminetetraacetic acid can decrease intra-mitochondrial ROS production and enhance cell viability. Antioxidant administration also reduces mitochondrial ROS production and cell loss, but this treatment does not synergize with Ca2+chelation.

Our results disclose novel intracellular pathways involved in PSY-induced death that may be exploited for therapeutic purposes to delay GLD onset and/or slow down its progression.

Image



T05-06A

Relationship between glial activation and neuroprotection induced by cannabinoid system modulation in the chronic MPTP mouse model

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Glial cells play an essential role in the development, function, and survival of neurons in the central nervous system. However, under pathological conditions, neuroinflammation has been associated with neuronal death. Neurodegenerative diseases show a marked glial immunoreactivity around the damaged area that has been related to the degenerative process. Our aim was to study whether there is a relationship between changes in glial activation and neuroprotection in an animal model of Parkinson´s disease. MPTP mice were treated for 5 weeks with drugs that modulate different elements of the cannabinoid system: JZL184, a monoacylglicerol lipase inhibitor; URB597, a fatty acid amino hydrolase inhibitor; cannabidiol (CBD), a GPR55 receptor antagonist; and abnormal-cannabidiol (abn-CBD), a GPR55 receptor agonist. At the end of each treatment, animal motor behaviour was evaluated and the status of the nigrostriatal pathway and glial changes were studied by histological techniques. JZL184 was the only treatment that showed a neuroprotective effect. Analysis of striatal astroglia activation in MPTP mice showed an increased immunoreactivity for GFAP that was exacerbated in MPTP mice that received JZL184; it did not change when mice were treated with URB597, and decreased after CBD and abn-CBD treatment. Three parameters of microglia morphology were analysed by stereology in Iba1 immunoreactive cells: cell density, cell body area and the extent of ramification length. MPTP did not induce significant changes in any of the measured parameters except in cell body area. JZL184 administration to MPTP mice induced a significant increase in all three microglial parameters. MPTP mice treated with URB597 showed a significantly decreased cell body area induced by MPTP when compared to control levels. Parkinsonian mice treated with abn-CBD and CBD showed a decrease in the microglial parameters when compared to MPTP mice. Modulations of elements of the cannabinoid system have different effects on neuroprotection of the nigrostriatal pathway of MPTP mice and induce different types of glial responses. Down-regulation of microglial activation to control level does not correlate with a neuroprotective phenotype in mice treated with abn-CBD and CBD. However, JZL-treated MPTP mice show an increase in astroglial and microglial activation that correlates with a neuroprotective phenotype. Our results suggest that chronic administration of JZL184 could induce neuroreparative glial activation that could be involved in the neuroprotective effect of the drug.

T05-07A

Reactive oxygen species (ROS) regulate ERK1/2 signaling and FGF expression in retinal gliosis

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Purpose: Gliosis is the response of glial cells within retinal tissue to injury. It can be beneficial in the short term, but can lead to scar formation, which contributes to blindness. Phosphorylation of ERK1/2 is considered to be a key event of gliosis, but the factors involved are poorly understood, particularly in the retina. Because reactive oxygen species (ROS) can inhibit phosphatases, thereby altering the phosphorylation events, we tested the hypothesis that ROS regulate the phosphorylation of ERK1/2 in gliosis.

Methods: Increases in pERK1/2 were detected using Western blotting and immunofluorescence in three models of retinal stress. Explanted murine retinas were used to identify the signaling partners of pERK1/2 via Western blotting in conjunction with inhibitors. Cell death was measured with terminal dUTP nick end labeling (TUNEL).

Results: We examined the phosphorylation status of ERK1/2 in two models of retinal degeneration and showed that up-regulation of pERK1/2 occurs. Using immunohistochemistry this localized to Muller glial cells. This was also shown in cultured retinal explants. There were increases in FGF, c-fos and pSTAT3 following explantation. Having demonstrated that retinal explants have a similar gliotic response to animal models of gliosis, specifically in terms of their transient up-regulation of pERK1/2 in Müller cells, we next sought to determine if ROS play a role in the regulation of phosphorylation events. Three inhibitors of ROS were used in the retinal explant system. NAC is a glutathione precursor and acts by altering the redox status of the cell. DPI and apopcyanin inhibits a flavo-containing proteins, including NADPH oxidase enzymes, a source of ROS. Doses were selected based on those typically used in previously studies. All three agents significantly reduce ROS level and decreased the levels of pERK1/2.

UO126 inhibits the upstream activators of ERK1/2, that is, MEK1 and MEK2. Treatment of explants with UO126 decreased pERK1/2 in the explant model, as expected. Finally, we wanted to establish the effect of pERK1/2 inhibition on retinal survival, given that its phosphorylation can trigger pro-survival and pro-death pathways. Cell death was examined using TUNEL. TUNEL positive cells were counted in each of the retinal layers and there was an increase in cell death at 48 hours with UO126 treatment. This shows that the increase in pERK1/2 in gliosis contributes to retinal cell survival.

Conclusions: This study show that ROS contribute to a pro-survival signaling pathway in retinal Müller cell gliosis.

T05-08A

Dysregulation of the S100B-RAGE pathway in the ALS-linked neuroinflammatory process

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The comprehension and control of the molecular mechanisms at the basis of astrocytic dysfunction in amyotrophic lateral sclerosis (ALS) are of fundamental importance to limit neuronal injury and disease progression. Most of the toxic effects exerted by astrocytes highlight the critical role of intracellular calcium in the activation of aberrant pathways that lead to astrocytosis. S100B is a Ca^{2+}-binding protein particularly present in astrocytes, that binds to and regulates the function of numerous target proteins. Moreover, S100B also has a role as neuroinflammatory mediator as it is secreted by astrocytes under pathological conditions and can display paracrine toxicity by binding to the receptor for advanced glycation endproducts (RAGE). During ALS progression S100B increases in astrocytes and motoneurons of patients and, in a rat model of the disease, S100B is augmented in “aberrant astrocytes”, characterized by lack of the glutamate transporter GLT-1 and increase in neurotoxic potential.

Our working hypothesis was that the induction of S100B in astrocytes, its release and its interaction with RAGE in motoneurons could represent an hazardous mechanism that takes place during ALS. Main objectives of our work were to investigate 1) if the expression of S100B protein and RAGE

change during the course of the disease in mouse and rat models of ALS and if they correlate with known markers of neuroinflammation; 2) if the expression of mutant SOD1 protein per se is sufficient to modify S100B levels in astrocytic cultures.

We observed that S100B levels and localization are modulated in the spinal cord gray and white matter and in the brain cortex of rat and mouse models of ALS. Contextually, we demonstrated a differential expression of RAGE subunits in SOD1-G93A-derived CNS tissues. Moreover, through an in vitro approach we showed that the overexpression of mutant SOD1 in astrocytic cell line is sufficient to increase the intracellular levels and release of S100B while it is not enough to induce a differential expression of RAGE. Our data demonstrate that the expression of mutant SOD1 interferes with the physiological expression of S100B and RAGE and reveal that in astrocytes S100B modulation is an early event related to the mere expression of mutant SOD1, while the dysregulation of RAGE might be a downstream phenomenon possibly requiring a more complex interplay between different cell types and pathways. Overall, these data would suggest that S100B might be a toxic mediator released by astrocytes in the ALS-linked neuroinflammatory process.

T05-09A

The role of the ERAD pathway in the physiology and disease of peripheral myelination

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P0, the most abundant glycoprotein in the peripheral myelin, is produced by Schwann cells and acts as an adhesive molecule in the myelin membrane. In humans the deletion of the serine 63 in the extracellular domain of P0 (P0S63del) causes the demyelinating CMT1B neuropathy, similarly manifested in transgenic S63del mice. The P0S63del protein is misfolded and retained in the ER, where it causes a chronic ER stress that triggers the unfolded protein response (UPR). In S63del nerves the UPR upregulates several ERAD genes, such as *derlins*. *In vivo* Derlins coimmunoprecipitate with the P0S63del protein and, importantly, the Schwann cell-specific ablation of Derlin-2 exacerbates the neuropathy suggesting an adaptive role of Derlins (and ERAD) in the disease. Moreover the Schwann cell-specific deletion of Derlin-2 *per se* is sufficient to cause a late demyelinating neuropathy indicating that an efficient ERAD is necessary to maintain myelin integrity possibly by preserving ER homeostasis.

T05-10A

Loss of acid sphingomyelinase activity causes changes in retinal microglial morphology and function in mice

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Background: Niemann-Pick disease type A and B are lysosomal storage disorders which are caused by loss of function mutations in the acid sphingomyelinase (aSMase) encoding gene causing symptoms like hepatosplenomegaly and rapid neurodegeneration. Intracellular loss of aSMase activity in aSMase-knockout mice leads to accumulation of sphingolipids in lysosomes, especially in macrophages. Because microglial cells are critically involved in retinal health we aimed to determine the consequence of aSMase deficiency in these immune cells.

Methods: Spectral-domain optical coherence tomography (SD-OCT) and infrared reflectance (IR) were used to characterize the integrity of the aSMase-deficient retina. Furthermore, retinal whole-mount and cross-section analysis of aSMase-KO mice were performed to determine the localization and immune status of retinal microglial cells by ionized calcium-binding adapter molecule 1 (Iba1) and

translocator protein (18kDa) (TSPO) immunostaining. Intracellular accumulation of lipids was determined by Nile red staining. Quantitative real-time PCR was used to measure inflammatory gene expression.

Results: Although SD-OCT showed no changes in architecture of retinal layers, IR fundoscopy revealed an increased number of hyperreflective spots in the ganglion cell layer of aSMase-deficient mice. Histological examination of Iba1-stained whole-mounts and retinal cross-sections showed an even distribution of microglia throughout the retina. However, we detected an increased microglial cell number with significantly enlarged cell bodies in the aSMase-deficient retina. Specifically, aSMase-decifient microglial cells showed enhanced proliferation and elevated expression of TSPO which points towards increased microglial reactivity. Additional qRT-PCR analysis revealed increased expression of the microglial reactivity markers CD68 and AMWAP in the aSMase-deficient retina. Microglial cells also displayed strong accumulation of lysosomal lipids as detected by Nile red.

Conclusion: These results suggest that aSMase deficiency and hence disturbed lipid metabolism affect the morphology and function of microglia in the retina. Elevated TSPO protein levels and increased expression of CD68 and AMWAP suggest inflammation in aSMase-deficient retinae.

T05-11A

Targeting myelin as potential interventional strategy for multiple system atrophy

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Multiple system atrophy (MSA) is a fast-progressing, rare neurodegenerative disease categorized as atypical parkinsonian disorder. During disease progression, alpha-synuclein (aSyn) aggregation leads to dysfunction of mature myelin-forming oligodendrocytes consecutively causing myelin loss and finally axonal as well as neuronal degeneration. Adult oligodendrocytes progenitor cells (OPCs) represent an endogenous source for replacement of dysfunctional oligodendrocytes. However, maturation of primary rat-derived OPCs is severely impaired in presence of intracellular aSyn. Reduction of intracellular aSyn levels restores maturation of OPCs suggesting a tight link between aSyn and OPC maturation. Moreover, we observe increased numbers of oligodendroglial cells despite a severe myelin loss in the MSA mouse model expressing aSyn under the myelin basic protein (MBP) promoter. Increased numbers of reactive Nkx2.2/Olig2-positve OPCs in MSA *post-mortem* tissue further indicate that OPCs differentiation and remyelination is severely impaired in MSA. Thus, we hypothesize that promoting remyelination in MSA exerts a beneficial effect on axonal and neuronal survival as well as the functional phenotype. Indeed, we demonstrate that myelin basic protein (MBP) expression in aSyn overexpressing primary OPCs is restored to control levels using the muscarine acetylcholine receptor antagonist Benztropine (BT) recently shown to exert a pro-myelinogenic effect. We further characterize the effect of BT administration in the MBP mouse model for MSA evaluating the potential use of BT in MSA. In addition, we employed an unbiased transcriptome analysis on primary OPCs overexpressing aSyn to define novel targets to promote OPC maturation and remyelination in the context of MSA.

T05-12A

Macroautophagy dysfunction in oligodendroglial cells reduces the internalization of α-synuclein

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Objective: The aim of the current study is to identify if autophagy dysfunction plays a role in the internalization and intracellular accumulation of exogenous α-synuclein (AS) in oligodendroglial cells in vitro.

Background: Multiple system atrophy (MSA) is an adult onset and progressive neurodegenerative disorder. The major hallmark of MSA is the presence of oligodendroglial cytoplasmic inclusions (GCIs) throughout the central nervous system. The main component of GCIs is the neuronal protein AS. The mechanisms involved in the formation of AS-positive intracytoplasmic inclusions are not elucidated to date. One possible mechanism could be an impairment of AS degradation by oligodendroglial cells, such as the autophagy or proteasome pathway.

Methods: The internalization of recombinant soluble and fibrillized AS (sAS, fAS) was investigated in the human oligodendroglial cell line MO3.13 using immunocytochemistry and western blot (WB). Internalization was confirmed by confocal microscopy. To trigger intracellular macroautophagy dysfunction the autophagy blockers bafilomycin A1 (BAF) and 3-methyladenine (3-MA) were used. Detection of reactive oxygen species (ROS) was accomplished using nitroblue tetrazolium chloride.

Results: Increased LC3B-II levels were measured upon blocking the fusion of the autophagosome with the lysosome. Internalization of both AS forms in oligodendroglia decreased significantly upon macroautophagy dysfunction compared to oligodendroglial cells treated with sAS/fAS only. Ubiquitin levels were reduced upon macroautophagy dysfunction compared to sAS/fAS only treatment. Macroautophagy dysfunction induced oxidative stress as analyzed by DJ-1 levels and ROS production.

Conclusion: Our results suggest that autophagy dysfunction leads to a reduced internalization of monomeric AS by oligodendroglia and is associated with decreased levels of ubiquitin. In subsequent experiments, genetic blocking of LC3B and LAMP2 in oligodendroglia will be performed to further understand the role of macroautophagy and chaperone-mediated autophagy in MSA.

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T05-13A

A critical role of neutrophil serine proteinase PR3-induced microglial activation on neuronal cell death

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Question: Neutrophils are among the first infiltrating cells into ischemic brain, possibly regulating delayed inflammation. Proteinase-3 (PR3), a serine proteinase, is released from neutrophil granules and is involved in the inflammatory process. PR3 is implicated in antimicrobial defense and cell death, but the exact role of PR3 in the brain is less well defined. In the present study, the effect of PR3 on glial activation was investigated. **Methods**: The experiments were performed on cultured rat microglia, primary cortical neurons and in vivo system. Glial activation was assessed by the intracellular level of reactive oxygen species and expression of inflammatory cytokines, and conditioned media from activated microglia by PR3 was used to assess neuronal death. Glial activation by PR3 was measured using microinjection of PR3 into the brain of rat. **Results**: Herein we show that PR3 increased the inflammatory responses including the intracellular ROS and proinflammatory cytokines in rat primary microglia. Conditioned media from PR3-treated microglia induced neuronal cell death in concentration dependent manner. Furthermore, microinjected PR3 into the striatum induced microglial activation. And treatment with neutralizing anti-PR3 monoclonal antibody and protease inhibitors ameliorated microglial activation both in rat primary microglia and in vivo model. **Conclusion**: These data indicate that PR3 is a direct modulator of glial activation and causes a neuronal death through the augmentation of inflammatory responses. We suggest that PR3 could be a new modulator of neuroinflammation, and PR3 blocking might be a novel therapeutic strategy for neuroinflammatory disease such as stroke and AD. **Acknowledgement:** This work was supported by Mid-career Researcher Program (2014R1A2A2A01003079) to Chan Young Shin, Basic Science Research Program (NRF-2010-0023638) to Kyoung Ja Kwon through the National Research Foundation of

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T05-14A

Impact of aging and Alzheimer's disease β-amyloid on microglial autophagy

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Autophagy (macro-autophagy) is a crucial cell survival mechanism that degrades and clears damaged organelles, invading pathogens and aggregated proteins within the cell. Furthermore, during energy shortage, it supplies the cell with the necessary amino acids by breaking down cell organelles. Dysregulation of autophagy is hypothesized to contribute to the development and progression of several diseases, including cancer and neurodegenerative diseases. In Alzheimer's disease (AD) diminished autophagy in neurons is thought to play a role in the accumulation of aggregated β amyloid (Aβ) proteins. While microglia, the CNS-resident innate immune cells, can turn into phagocytes, their function is impaired in AD (Krabbe et al., PLoS One 2013). Beclin-1 (ATG 6), a key autophagic protein, recently has been shown to influence phagocytosis of Aβ in vitro in the microglia cell line BV2, and to be decreased in microglia derived from human AD patients (Lucin et al., Neuron 2013). The link between phagocytosis and autophagy, including key molecules such as Beclin-1, in microglia was assessed by western blot and immunohistochemistry. We used whole brain lysates and compared them to microglia isolated from two distinct strains of transgenic mice depositing Aβ and wild type mice of various ages. Our data provide a detailed microglia signature of autophagy proteins in response to aging and Aβ.

T05-15A

Gene delivery targeted to myelinating cells to treat inherited neuropathies

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Mutations in the *GJB1* gene encoding the gap junction protein connexin32 (Cx32) cause X-linked Charcot Marie Tooth disease mainly through cell-autonomous loss of function in myelinating Schwann cells. In order to establish a gene delivery method targeting myelinating cells of the peripheral nervous system (PNS) we generated a lentiviral vector containing the *GJB1* gene along with IRES-EGFP as a reporter gene under the control of the myelin protein zero (Mpz) promoter, which is specific for Schwann cells. A mock vector lacking *GJB1* was first delivered by a single lumbar intrathecal (L5-6) injection in 2 month-old wild type mice in order to assess vector spreading and EGFP expression, while the full vector was delivered intrathecally in 2 month-old Cx32 knockout (Cx32 KO) mice in order to assess Cx32 expression. EGFP expression was detected 2 to 16 weeks post-injection in posterior and anterior lumbar roots, DRGs, femoral motor nerves, in proximal, middle and distal parts of the sciatic nerve, as well as in intramuscular nerves of the quadriceps muscle indicating vector spread through the roots to the peripheral nerves. EGFP expression rates increased over time and varied among different PNS areas with highest levels in the sciatic nerve (56.5±3.51%), followed by the femoral nerve (48±5.03%), anterior lumbar roots (42.5±2.39%), DRGs (39.3±3.93%) and posterior lumbar roots (34.2±2.76%). EGFP expression was also confirmed by immunoblot analysis. Intrathecal injection of the full vector in Cx32 KO mice resulted in Cx32 expression in lumbar roots as well as in sciatic and femoral teased nerve fibers, and in the trigeminal nerve, correctly localized at paranodal myelin loops. Cx32 expression was further confirmed by immunoblot analysis and by reverse transcription-PCR followed by human and mouse transcript-specific digest. Sciatic nerves of injected Cx32 KO mice expressed only the human *GJB1*, while control WT mice expressed only the mouse *Gjb1*. There was no evidence of inflammatory response following intrathecal vector delivery and gene expression. In conclusion, we report a non-invasive, clinically relevant method for gene delivery with a single lumbar intrathecal injection that provides long lasting, widespread and targeted reporter and

disease-related gene expression in PNS myelinating cells. This approach can be potentially used for clinical applications and should be further studied.

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T05-16A

Consequences of the chronic activation of hemichannels in astrocytes of a murine model of Alzheimer's disease

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In Alzheimer's disease (AD), astrocyte properties are modified but their role in the pathology only starts being explored. We have previously shown that the expression of connexins (Cx), proteins forming gap junction channels and hemichannels, increased in astrocytes close to amyloid (Ab) plaques in brains from AD patients and two different strains of AD models (APP/PS1 mice). Since, in brain slices, acute Ab application triggers astroglial Cx43 hemichannel activity leading to neuronal degeneration, we have investigated hemichannel function in the hippocampus of 9 month old $APP_{swe}/PS1_{dE9}$ mice that exhibit Ab plaques. Ethidium bromide (EtBr) uptake in acute brain slices was used as index of hemichannel activity and was quantified in GFAP-immunostained astrocytes. Since in addition to connexins, another family of membrane proteins named pannexins (Panx) can form hemichannels, their respective contribution to the hemichannel activity was analyzed by using blockers of each family.

We have shown that hemichannels were activated in all hippocampal astrocytes with a higher EtBr uptake in reactive astrocytes contacting Ab plaques. While Cx43 was the major hemichannel contributor in the overall population of astrocytes, a minor Panx1 component was solely identified in astrocytes contacting plaques. Distinct pathways were involved in Cx and Panx hemichannel activation. Inflammation triggered Panx1 hemichannels since their activity was inhibited by minocycline or TNFa+IL1b antagonists, but did not affect Cx43 hemichannels. The latter were triggered by the high astroglial $[Ca^{2+}]_i$ measured in fluoro-4AM loaded slices. Indeed, reducing resting $[Ca^{2+}]_i$ to physiological level by blocking mGluR5, purinergic or IP3/RyR receptors inhibited Cx43 hemichannel activation but not Panx1. Then we have shown that astroglial hemichannel activation allowed for ATP and glutamate release and contributed to maintain a high $[Ca^{2+}]_i$ placing astrocytes in the center of a vicious circle. Finally we have shown that the lack of astroglial Cx43 alleviated neuronal damage in $APP_{swe}/PS1_{dE9}$ mice. In $APP_{swe}/PS1_{dE9}$ with an astroglial targeted knocking out of Cx43 gene, the level of oxidative stress in pyramidal neurons and the abundance of neuritic dystrophies associated with Ab plaques were strongly reduced. Hence, blocking astrocyte hemichannels could represent an alternative therapeutic strategy in AD.

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T05-17A

Prolonged astrocytes dysfunction and dopaminergic neurons degeneration cause small changes in mitochondrial complex I and IV activity and supercomplexes assembly in substantia nigra

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The degeneration of dopaminergic neurons in substantia nigra (SN) is underlying cause of movement disorder observed in Parkinson's disease (PD). The role of astrocytes in PD is still unrecognized. It is probable that prolonged dysfunction of astrocytes could increase dopaminergic neurons vulnerability.

Our aim was to investigate if prolonged metabolic inhibition of astrocytes would influence mitochondrial Cx I and IV function, their assembly into supercomplexes and how it would modify response to selective degeneration of dopaminergic neurons in the rats brain.

Rat model of selective nigrostriatal dopaminergic system degeneration was induced by intracerebral injection of 6-hydroxydopamine (6-OHDA) into medial forebrain bundle. Astrocytes metabolic dysfunction was induced by 7-days constant infusion of fluorocitrate (FC) into SN using osmotic minipumps. Animals were sacrificed 1 or 4 weeks after operation. In-gel enzymatic activity measurements of mitochondrial complex I and IV we performed on solubilized crude mitochondrial membrane fraction from SN.

Inhibition of astrocytes metabolism by FC caused tendency to decreased performance and amount of complex I in supercomplex $I_1III_2IV_1$ and of individual form I_1 and decreased performance and amount of individual complex IV_1, directly after 7 days infusion. Lesioning dopaminergic neurons didn't show statistically significant changes in this experiment. Double toxicity of 6-OHDA and FC also caused decreased performance in complex I and IV and this effect was stable even 4 weeks after operations and FC discontinuation for 3 weeks. At this time-point also specific activity of complex IV was significantly decreased.

This is the first report to prove that prolonged dysfunction of astrocytes influences dopaminergic system cells oxidative phosphorylation system complexes performance with regard to its supercomplexes architecture. Since changes in this system are so small and difficult to prove statistically even after loss of 33% to 61% of neurons - it would suggest extensive adaptive possibilities. Interestingly decreased parameters for complex I and IV function correlated more with distortion of astrocytes function than with neuronal degeneration.

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T05-18A

Expression of PDGFR-β positive NG2 cells in the hippocampus after status epilepticus

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Platelet-derived growth factor receptor β (PDGFR-β) is a tyrosine kinase receptor and a component of extracellular matrix. PDGFR-β is involved in proliferation and migration of pericytes, fibroblasts and vascular smooth muscle cells, and neuronal survival. We hypothesized that PDGFR-β expression contributes to hippocampal plasticity induced by epileptogenic brain insult.

Status epilepticus (SE) was induced in adult male C57BL/6JOlaHsd mice by intrahippocampal injection (60 nl) of 10 mM kainic acid (KA). Control animals were injected with saline. Mice were sacrificed at 1 d, 4 d, or 7 d post-KA for immunohistochemical assessment of expression of PDGFR-β in the hippocampus. The total number of PDGFR-β positive cells was estimated using unbiased stereology. The identity of PDGFR-β positive cells was characterized using double-immunofluorescence with either NG2 or GFAP antibodies.

In the normal hippocampus, PDGFR-β positive cells were relatively evenly distributed in different subfields. After SE, distribution of PDGFR-β positive cells changed remarkably. Their total number increased ipsilaterally in the hilus (control 139 ± 14 *vs.* SE 4 d 248 ± 35 or SE 7 d 314 ± 41; both $p < 0.05$) and infrapyramidal and suprapyramidal layers of the CA3 (control 709 ± 157 *vs.* SE 7 d 1424 ± 169; $p < 0.05$) whereas in the CA1 the number of PDGFR-β positive cells was reduced (control 560 ± 42 *vs.* SE 1 d 326 ± 37; $p < 0.05$) but returned to control level by 4 d post-SE (SE 656 ± 101, $p > 0.05$). Contralaterally, they remained evenly distributed throughout the 7 d follow-up. PDGFR-β positive cells were characterized by large somata and highly ramified processes comparable to the morphology of NG2 cells. Further analysis with confocal imaging confirmed that all PDGFR-β positive cells were either NG2 cells or astrocytes. One hundred-% of NG2 cells and about fifty-% of astrocytes expressed PDGFR-β in saline or KA-treated animals.

Our data show that PDGFR-β is predominantly expressed in NG2 cells. After epileptogenic injury they accumulate into hippocampal subfields with most severe damage, suggesting their role in neuroprotection.

T05-19A

Antioxidant effect of an alpha-MSH analogue in primary astrocytes cultures

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Inflammatory processes in the central nervous system (CNS) contribute to the development of neurodegenerative disorders and oxidative stress appears to be connected with the loss of neurons during the progression of these diseases. Astrocytes are the cell type with the most antioxidant capabilities within the CNS and they supply neurons with glutathione (GSH) precursors. Melanocortins are neuropeptides with proved anti-inflammatory and neuroprotective properties. Previously, we have shown that alpha-melanocyte-stimulating hormone (alpha-MSH) exerts an anti-inflammatory action through MC4 receptors in astrocytes. Considering the importance of developing new therapeutic strategies focused on the regulation of antioxidant mechanisms, we decided to evaluate melanocortins' antioxidant effect in cultured rat primary astrocytes using NDP-MSH, a synthetic analogue of alpha-MSH. Primary cultured rat astrocytes were incubated for 24 h with NDP 0.1 μM and intra and extracellular GSH levels were evaluated using monochlorobimane. NDP-MSH increased extracellular GSH levels by 21.2% ($p<0.001$) while it did not change intracellular GSH levels. Next, we determined the enzymatic activity of gamma-glutamate-cysteine ligase (gamma-GCL), the rate-limiting enzyme in GSH synthesis. Exposure of astrocytes to NDP-MSH increased gamma-GCL activity in a dose-dependent manner ($p<0.01$). However, treatment with NDP-MSH for 6 h did not modify gene expression of either catalytic (GCLc) or modifier (GCLm) subunits of gamma-GCL, assessed by real time-PCR.

Nuclear factor erythroid 2-related factor 2 (Nrf2) is considered the primary cellular defense against cytotoxicity caused by oxidative stress, by modulating the expression of antioxidant enzymes like heme oxygenase 1 (HO-1). We observed nuclear translocation of Nrf2 by immunocytochemistry in astrocytes treated with NDP-MSH for 6 h. Moreover, HO-1 mRNA levels were also increased upon NDP-MSH treatment.

Finally we tested superoxide dismutase (SOD) activity through the epinephrine auto-oxidation technique. NDP-MSH increased SOD activity by 52.7% ($p<0.05$).

In summary, NDP-MSH might exert an antioxidant effect in primary cultured astrocytes by means of modulation of gamma-GCL and SOD activities, stimulation of GSH release and induction of Nrf2 nuclear translocation and HO-1 gene expression, thereby contributing to the neuroprotective properties of melanocortins.

T05-20A

Calcineurin-mediated deregulation of astroglial Ca^{2+} signaling by β-amyloid: implications for neuronal dysfunction in Alzheimer's disease

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The amyloid hypothesis of Alzheimer's disease (AD) suggests that soluble β-amyloid (Aβ) initiates pathogenic cascade, involving deregulation of Ca^{2+} homeostasis, which leads eventually to neurodegeneration. Astrocytes may mediate effects of Aβ on neuronal dysfunction. Here we dissect a cascade of signaling events by which Aβ deregulates astroglial Ca^{2+} signaling: (i) 100 nM $Aβ_{42}$ leads

to an increase in cytosolic calcium after 4-6 h of treatment; (ii) increased Ca^{2+} leads to activation of calcineurin (CaN), which in turn (iii) directly activates NFAT to up-regulate IP_3R1, and (iv) via interaction with Bcl10 and degradation of IkBα activates NF-kB to up-regulate mGluR5 and IP_3R2. IP_3R1-mediated Ca^{2+} release and IP_3R1 protein were augmented in the hippocampal, but not in the entorhinal cortex astrocytes form 3xTg-AD mice. We also show, that mGluR5 staining is augmented in hippocampal astrocytes of AD patients in proximity of Aβ plaques and co-localized with accumulation of the p65 NF-kB subunit and increased staining of CaNAα. Next, we show that store-operated Ca^{2+} entry (SOCE) was augmented in $Aβ_{42}$-treated astrocytes due to up-regulation of TRPC1 and TRPC4. Increased SOCE was also found in astrocytes from 3×Tg-AD mice, suggesting a link to the AD pathology. To assay the specificity of the Aβ effects, we compared them with the effects of pro-inflammatory agents such as TNFα, Il-1β and LPS. In contrast to Aβ, pro-inflammatory cytokines and LPS significantly reduced expression of mGluR5, IP_3R1 and IP_3R2, suppressed SOCE and down-regulated SOCE-related genes in hippocampal astrocytes. Interestingly, the degradation of IkBα by TNFα, Il-1β and LPS did not depend on CaN activation. Finally, using astrocyte-neuronal co-culture system and conditioned medium transfer, we show that $Aβ_{42}$-exposed astrocytes, as well as astrocytes form 3xTg-AD mice produce degeneration of dendritic spines and reduction of MAP2, PSD95 and Syn38 in cultured neurons, the effects blocked by CaN inhibitor. Taken together, our data suggest that in astrocytes Aβ produces unique pattern of Ca^{2+} signaling remodeling that depend on CaN activation and which may be relevant to the AD neuropathology.

T05-21A

Microglial lipid markers by using maldi-imaging mass spectrometry in a basal forebrain cholinergic lesion model

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The basal forebrain cholinergic neurons (BFCN) that control learning and memory processes, are damaged in Alzheimer´s disease (AD). Neuroinflammation, astrogliosis and microglial activation are common features in several neurodegenerative disorders including AD. 192IgG-saporin (SAP), an immunotoxin directed against the Low-Affinity Nerve Growth Factor Receptor ($p75^{NTR}$), specifically eliminates BFCN. The present study analyzes the glial response in the adult rat model of specific cholinergic lesion at the *nucleus basalis* of Meynert (nbM). The density of BFCN and the presence of cannabinoid type-1 receptor (CB_1) and microglia were labeled by immunofluorescence ($p75^{NTR}$, CB_1 and Iba-1 or OX-42). Previously, some parameters of learning and memory processes were evaluated using passive avoidance (PA, for fear-conditioned memory) and Barnes maze (BM, for spatial memory) tests. The anatomical distribution of the lipid composition in brain slices was obtained by using Matrix-Assisted Laser Desorption Ionization - Imaging Mass Spectrometry (MALDI-IMS). Immunofluorescence studies revealed the loss of approximately 80% of BFCN and a high density of CB_1 immunoreactivity accompanied by proliferation of activated microglia compared to the control group (VEH). Behavioral studies showed cognitive impairment in both, PA test ($p<0.001$ VEH *vs* SAP), and BM test (VEH 5.71 ± 0.61s *vs* SAP 14.91 ± 4.49s; $p<0.05$), which correlated with the reduction in the density of BFCN ($p75^{NTR}$-ir, VEH *vs* SAP; $r^2=0.51$, $p<0.05$). Surprisingly, specific complex lipids including different species of glycerophospholipids, such as phosphatidylcholines, and sphingolipids, such as sphingomyelins and sulfatides were found modified in the injured area accompanied by an up-regulation of activated microglia. The BFCN lesion model induces neuroinflammatory processes and microglial activation, probably modifying its lipid composition as is demonstrated by using the combination of immunofluorescence and MALDI-IMS techniques. The specific lipid changes are probably indicating not only a cell-specific lipid profile but also the glial response to the cholinergic damage. Further studies would help to elucidate the role of complex lipids in neurodegenerative processes to identify new therapeutic targets.

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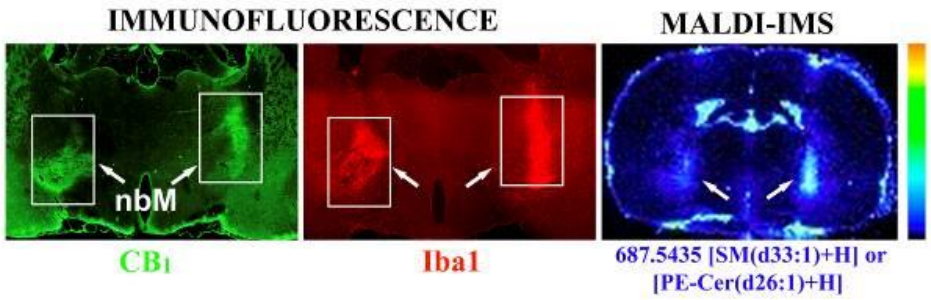
Image



T05-22A

IFNß treatment as a therapy targeting microglia in a murine model of retinal degeneration

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Purpose: Age-related macular degeneration (AMD) is a leading cause of vision loss in the elderly. Typical hallmarks of AMD are chronic activation of the innate immune system and reactive microglial cells in the retina. Here, we analyzed the role of interferon-beta (IFNß) signaling and the effect of IFNß therapy on microglial activation and choroidal neovascularization in a murine model of AMD-like retinal damage.

Methods: Laser-rupture of Bruch's membrane was used as a murine model for AMD. Retinal inflammation and choroidal neovascularization (CNV) were analyzed in IFN-alpha/beta receptor knockout ($IFNAR^{-/-}$) mice, IFNß-treated C57BL6/J mice and C57BL6/J wild type controls using fundus fluorescein angiography (FFA), lectin staining and optical coherence tomography (OCT). Microglial morphology in laser-induced lesions was analyzed by Iba1 and Tspo staining of flatmounted retinas and retinal pigment epithelia (RPE).

Results: Laser-induced lesions in $IFNAR^{-/-}$ animals showed increased vessel leakage as well as CNV compared to control animals, indicating that $IFNAR^{-/-}$ deficiency enhanced inflammation. In contrast, IFNß-treated animals showed reduced vessel leakage and CNV compared to untreated controls. OCT-analysis of IFNß-treated and untreated wild type mice 7 and 14 days after induction of the retinal damage revealed diminished edema formation in IFNß-treated animals. Immunohistological analysis of flat-mounted laser-damaged retinas displayed both, a higher number and a longer presence of activated microglial cells at the sites of damage in $IFNAR^{-/-}$ mice compared to controls. The amount of activated microglia cells in IFNß-treated animals was lower than in respective control groups. Iba1 staining of flatmounted RPE revealed microglial cells in the subretinal space of laser-treated $IFNAR^{-/-}$ and C57BL6/J wild type animals but not in laser- and additionally IFNß-treated mice.

Conclusion: Knockout of IFNAR leads to enhanced retinal inflammation and microglial reactivity. In contrast, IFNß therapy significantly prevented vessel leakage, CNV and microglial activation. We conclude that IFNß signaling dampens microglial reactivity and is a protective mechanism in retinal degeneration.

T05-24A

Dichloroacetate modulation of mitochondrial function reduces toxicity to motorneurons in aged glia from Amyotrophic Lateral Sclerosis rat model

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Amyotrophic Lateral Sclerosis (ALS) is a neurodegenerative disease characterized by motorneuron loss and reactive gliosis. Neonatal astrocytes from ALS-linked mutated superoxide dismutase-1 transgenic rats (SOD1G93A) exhibit oxidative stress and mitochondrial dysfunction associated to neurotoxic activity for non-transgenic (nTg) motorneurons which can be prevented by the metabolic modulator dichloroacetate (DCA), antioxidants or by stimulating antioxidant defences. Aged glia isolated from adult symptomatic SOD1G93A rats ("AbAs" for Aberrant Astrocytes) display a highly proliferative and neurotoxic phenotype. Aim: to analyze whether mitochondrial activity and oxidative stress play a role in AbAs neurotoxicity. Methods: Established AbAs cultures (passages 6-10) were treated with DCA (5mM) and mitochondrial function, proliferation and neurotoxic activity of its conditioned media to purified embryonic motorneurons were analyzed and compared to neonatal SOD1G93A and nTg astrocytes. Results: Confocal microscopy analysis of mitotracker revealed fragmented mitochondria in AbAs associated to lower mitochondrial oxygen consumption compared to neonatal astrocytes. DCA exposure improved AbAs mitochondrial function, reduced proliferative rate and, importantly, decreased motorneuron toxicity of AbAs conditioned media. Furthermore, DCA treatment elicited an increase in free radical production as quantified by MitoSox probe fluorescence and hemeoxygenase-1 (HO-1) levels detected by westerblot and immunofluorescence. Current experiments are undergoing to analyze whether oral administration of DCA to SOD1G93A rats reduce glial proliferation and up-regulate HO-1 expression in the spinal cord. Conclusions: Our results support that mitochondrial dysfunction is related to aged glia neurotoxicity to motorneurons and indicates that stimulation of antioxidant defences may be involved in the ability of DCA to modify the neurotoxic AbAs phenotype. Understanding the mechanisms underlying the beneficial effects of DCA in disease progression will contribute to clarify the therapeutic potential of targeting glial mitochondrial function in ALS.

T05-25A

Reactive astrocytes secrete exosomes that induce motor neuron death. Implications for ALS

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Reactive astrogliosis is a common response of astrocytes to brain injuries as trauma, ischemia, infection, and neurodegeneration. In the neurodegenerative disease amyotrophic lateral sclerosis (ALS), reactive astrocytes undergo morphological changes and alterations in gene expression that modify their neurotrophic properties and instead induce the death of surrounding motor neurons. In different models of the disease, astrocyte conditioned media exhibit toxicity towards motor neurons but the exact nature of the neurotoxic agent in the conditioned media is not well understood. Most ALS cases (90-95%) are sporadic, but 2% of cases are associated to mutations in Cu/Zn Superoxide Dismutase 1 (SOD1). Recently, Basso et al. demonstrated that exosomes isolated from astrocytes derived from SODG93A transgenic mice transfer mutant SOD1 to spinal neurons and induce selective motor neuron death in co-culture with astrocytes, suggesting that exosomes may contribute to disease pathology. To further analyze whether the neurotoxicity was linked to the mutant SOD expression we compared the effects of exosomes derived from SODG93A-expressing rat astrocytes and IL-1B/LPS stimulated wild-type astrocytes on the survival of isolated spinal motor neurons. Exosomes derived from both types of astrocytes were characterized by electron microscopy and analysis of their differential protein content. Purified embryonic motor neurons were incubated with the different fractions of astrocyte-conditioned media: Both SOD1G93A-expressing and IL1B/LPS-stimulated astrocyte conditioned media were directly toxic for motor neurons even in the presence of growth factors. Purified exosomes from both types of astrocytes were sufficient to induce the death of motor neurons while the exosome-free astrocyte conditioned media did not induce motor neuron death. These results support the hypothesis that exosomes play a significant role in ALS spreading, through

other mechanisms besides the familial-linked mutated SOD. We are currently searching for candidate molecules responsible for the exosome-mediated neurotoxicity in these different models of astrocyte reactivity.

T05-27A

Glia in Prion diseases

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Pathogenesis of prion diseases is mostly unknown and as consequence, to date it has been impossible to develop preventive and/or paliative treatments against them. The host protective response has been taken very little into account, or completely ignored, in relation with this group of diseases. Although gliosis constitutes one of the histopathological changes typically found in the brains of affected cases joint to neuronal degeneration and deposit of pathological prion protein, a little or no relevance has been certainly pinpointed to the components of glial population in the characteristic neurodegeneration of these diseases. To our knowledge, the absence of comparative studies at cellular level on these processes, specially related with host protection, has widely delayed the understanding of prion pathologies and therefore, the finding of some efficient treatment. This study is based on an exhaustive assessment of the distribution and morphology of the astroglial and microglial cells as components of the host protective system to determine how their interaction with pathological prion protein could influence on the development and propagation of the disease. In order to achieve this aim a morphological approach has been developed on samples belonging to different Creutzfeldt-Jakob disease in comparison with control healthy cases. The actual significance of gliosis in prion diseases is intended to be finally explained. In case it is confirmed the design of new therapeutic alternatives would be allowed. But moreover, all knowledge provided in the frame of this approach result especially relevant, not only for prion diseases, but also for other neurodegenerative diseases such as Alzheimer or Parkinson, due to the close association between them, which is gaining more supporters since they seem to share molecular basis and mechanisms of propagation. Acknowledgments.- This work was funded by a grant from the University of Zaragoza (UZ2014-BIO-4)

T05-28A

Activation of the S1P receptor attenuates psychosine-induced demyelination and astrocyte dysfunction

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Krabbe disease is a rare autosomal recessive neurodegenerative disorder affecting 1:100,000 births. This illness is rapidly progressing, appearing within the first three to six months of life and is usually fatal by the age of two years. Krabbe disease is caused by a deficiency in the lysosomal enzyme galactocerebrosidase (GALC), which results in the accumulation of a toxic metabolite in the brain termed psychosine. The build-up of psychosine is believed to be the main pathogenic agent in Krabbe disease resulting in widespread oligodendrocyte cell death and demyelination. To date, most studies have focused on the toxic effects of psychosine on oligodendrocytes, however little is known about the effects of psychosine on astrocytes. Astrocytes, the most abundant cell type in the brain, have many important functions such as protection and support of neurons and oligodendrocytes. Astrocytes also play key roles in regulating metabolic and ion homeostasis in the CNS. Importantly, astrocytes express S1P receptors (S1PR) which are targets for the first oral therapy developed for multiple sclerosis, FTY720 (Fingolimod). S1PRs have various roles in the CNS, including astrocyte migration, oligodendrocyte survival, and neurogenesis. More recently, S1PRs have been found to play a role in inhibiting the release of pro-inflammatory cytokines from glial cells. Here we investigate the effect psychosine has on human and rat astrocyte survival and on the release of pro-inflammatory cytokines from mouse astrocytes. In addition we investigate the effect psychosine has on the myelination state of mouse cerebellar slices. Importantly we demonstrate the protective effects pFTY720 has against both the psychosine induced cell-toxicity and the psychosine induced demyelination of cerebellar slices.

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T05-29A

Effect of astrocytes prolonged dysfunction on dopaminergic system degeneration and functional compensation of motor deficits, in relation to early Parkinson's disease

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The degeneration of dopaminergic neurons in substantia nigra (SN) is underlying cause of movement disorder observed in Parkinson's disease (PD). The role of astrocytes in PD is still unrecognized. It is probable that prolonged dysfunction of astrocytes could increase dopaminergic neurons vulnerability. Our aim was to investigate if prolonged dysfunction of astrocytes would *i)* induce degeneration of dopaminergic neurons in SN, *ii)* influence neurodegeneration caused by selective anti-dopaminergic toxin, *iii)* have impact on compensatory capacity.

Rat model of selective nigrostriatal dopaminergic system degeneration was induced by intracerebral injection of 6-hydroxydopamine (6-OHDA) into medial forebrain bundle. Astrocytes dysfunction was induced by 7-days constant infusion of fluorocitrate into SN using osmotic minipumps. Animals were sacrificed 1 or 4 weeks after operation. Behavioral analysis of locomotor activity was performed at 4, 6 and 27 day after operation in automated cages, for 18 hours. HPLC-EC analysis of DA, its metabolites and turnover rates and stereological counting of neurons in SN were performed.

Decreased locomotor activity was visible in lesioned animals 4 days after 6-OHDA injection. Disrupting astrocytes function also caused small decrease in behaviour and it enhanced disability of lesioned animals. Already 6 days after the operation the effect of lesion was minimized and disappeared at 27th day, proving functional compensation despite progressing degeneration. Double lesioned animals recoverded only to some degree.
Selective degeneration of dopaminergic neurons in SN after 1 week was progressing until 4 weeks after lesioning. Astrocytes dysfunction provoked degeneration of dopaminergic neurons density and accelerated (but not enhanced) their degeneration caused by 6-OHDA.
Dopamine (DA) and metabolites levels in STR and SN decreased one week after lesioning and progressed after 4 weeks. Dysfunction of astrocytes decreased DA level in STR but significantly increased its metabolism in SN. This effect was reversed after treatment discontinuation at 4 weeks. Glial dysfunction enhanced DA and metabolites decreases in STR caused by 6-OHDA after one week but no further aggravation with time was detected due to increased DA turnover.

This is the first report to prove that prolonged dysfunction of astrocytes influences dopaminergic neurons survival, metabolism and compensatory potential.

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T05-30A

Expression of Kir4.1 channel in spinal cord oligodendrocytes of the ALS rat model

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Amyotrophic lateral sclerosis (ALS) is a progressive and fatal neurodegenerative disease affecting lower and upper motor neurons. Previous research suggested that glia plays important role in disease progression and death of motor neurons. Glial cells maintain equilibrium ion concentration and provide support for neuronal functioning. One of the most prominent roles of glial cells is the potassium uptake and regulation of its physiological concentration in the extracellular environment. The key role in maintaining this function is played by the inwardly rectifying potassium channel, Kir4.1 expressed in

astrocytes and oligodendrocytes surrounding motor neurons. We examined the expression levels of Kir4.1 in oligodendrocytes in the spinal cord gray matter of the $hSOD^{G93A}$ rat model of ALS. Double immunolabeling of Kir4.1 and CNPase was used to investigate colocalization and expression of these proteins in the cervical and lumbar regions of the spinal cord in end phase ALS. Reduced immunoreactivity of Kir4.1 and CNPase was observed in the ventral horn where the disease is known to be most severe. ALS oligodendrocytes labeled with the anti-CNPase antibody showed changed organization, morphology and signs of degeneration. In addition, Pearson and Manders colocalization coefficients showed a decrease in Kir4.1 and CNPase signal colocalization in the ventral horn. The finding of a reduced Kir4.1 expression in oligodendrocytes underlines the role of these glial cells in the mechanism of motor neuron death in ALS.

T05-31A

Modulation of RAS activity by estrogen takes place in both astrocytes and microglia. Implications in dopaminergic cell degeneration

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Introduction: The neuroprotective effects of menopausal estrogen replacement therapy (ERT) in Parkinson's disease have not been clarified. The loss of estrogen (E2) following ovariectomy (OVX) increases Renin Angiotensin System (RAS) activity, neuroinflammation, oxidative damage, and dopaminergic (DA) neuron degeneration. ERT induces RAS inhibition and neuroprotection when administered shortly after OVX. We studied the possible existence of a critical period for E2 neuroprotection, the interaction of ER and RAS activity in DA neuroprotection and the cellular target of E2-mediated neuroprotection.

Materials and Methods: Ovariectomized rats treated with the DA neurotoxin 6-hydroxydopamine (6-OHDA) were used to determine the neuroprotective effect of ERT administered at different times (0, 6, and 20 weeks) after OVX. In a second series of experiments, rats were used to study the effects of ERT on RAS activity, markers of NADPH-oxidase activation and neuroinflammation and levels of ER-a and ER-b in the substantia nigra (SN).

The in vitro experiments were carried out with primary (neuron-glia) mesencephalic cultures and cultures of primary mesencephalic astrocytes, MES 23.5 DA neuron cell line, and N9 microglial cell line. Laser confocal microscopy was used to study location of ER-a and ER-b in different cell types. To study the role of DA neurons, astrocytes, and microglial cells in the interaction of ER and RAS activity for neuroprotection, cultures were treated with the DA neurotoxin 1-methyl-4-phenylpyridinium (MPP^+) and/or Angiotensin (AII) and agonists of ER-a or ER-b.

Results: E2 induced significant protection against 6-OHDA-induced DA degeneration when administered immediately or 6 weeks, but not 20 weeks after OVX. In the SN, OVX decreased levels of ER-a and increased RAS activity, NADPH-oxidase activity, and expression of neuroinflammatory markers, which were regulated by E2 administered immediately or 6 weeks but not 20 weeks after OVX. Interestingly, treatment with AII receptor antagonists after the critical period induced neuroprotection. In cultures, treatment with MPP^+ induced an increase in astrocyte-derived angiotensinogen and DA neuron death, which were inhibited by ER-a agonists. In microglial cells, ER-b agonists inhibited the AII-induced increase in inflammatory markers.

Conclusions: The results suggest that there is a critical period for the neuroprotective effect of E2 against DA cell death, and that E2 modulates RAS activity and subsequent neuroinflammatory responses via both astrocytes and microglia.

T05-32A

Mitochondrial division inhibitor 1 induces mitochondrial and endoplasmic reticulum stress that exacerbates excitotoxic oligodendrocyte death

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Mitochondrial fusion and fission events in mammals are controlled by several GTPases of the dynamin family and play a critical role in the regulation of mitochondrial homeostasis. In particular, mitochondrial fission, which is mediated by cytosolic dynamin related protein 1 (Drp1), is essential for removal of damaged mitochondria but may contribute to apoptosis as well. Indeed, Drp1 inhibition or down-regulation provides neuroprotection in models of neurodegeneration and cerebral ischemia. However, the effects of Drp1 inhibition in oligodendrocytes have not been described yet. In the present study, we have analyzed the link between Drp1-mediated mitochondrial fission and intracellular Ca^{2+} homeostasis in optic nerve-derived oligodendrocytes. Using time-lapse confocal imaging in oligodendrocytes expressing mitochondria-targeted fluorescent proteins we found that sublethal AMPA receptor activation triggered mitochondrial fission, which was inhibited by mitochondrial division inhibitor 1 (Mdivi-1, 50 μM, 1 h), a selective inhibitor of Drp1. However, longer exposures to Mdivi-1 alone dramatically reduced oligodendrocyte viability. Since Drp1 is important for mitochondrial quality control, we next analyzed whether Mdivi-1 induced mitochondrial dysfunction. Rhodamine-123 imaging revealed a strong and dose-dependent mitochondrial depolarization after incubation of oligodendrocytes with Mdivi-1 for one hour, which was followed by reactive oxygen species (ROS) production and ultimately caspase-3 activation. Mdivi-1 also compromised endoplasmic reticulum (ER) homeostasis, characterized by ER Ca^{2+} depletion and ER stress induction, and potentiated thapsigargin-induced cell death. On the other hand, toxic insults with AMPA plus cyclothiazide (CTZ) resulted in mitochondrial swelling rather than fission. Mdivi-1 did not prevent excitotoxic mitochondrial swelling whereas cell death was exacerbated. Finally, Drp1 overexpression in oligodendrocytes did not rescue from Mdivi-1-, thapsigargin- and AMPA-induced cell death, indicating that Mdivi-1 activates a deleterious Drp1-independent mechanism. In summary, our results provide evidence of Drp1-mediated mitochondrial fission during physiological activation of ionotropic glutamate receptors in oligodendrocytes, and uncover a deleterious and Drp1-independent effect of Mdivi-1 on mitochondrial and ER function that turns these cells more vulnerable to intracellular Ca^{2+} homeostasis disruption.

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T05-33A

Indications for gliosis in Niemann-Pick type C1 patient-specific iPSC derived glia cells

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Niemann-Pick type C1 (NPC1) is a rare progressive neurodegenerative disease, which is caused by a mutation in the NPC1 gene. In this lysosomal storage disorder the intracellular transport and sequestration of several lipids like cholesterol is severely impaired resulting in their accumulation in the late endosomes and lysosomes. The neurological manifestation of the disease is caused by dysfunction and death of neurons, astrocytes and microglia. Gliosis was described in a commonly used NPC1 mouse model and over-actived glial cells are discussed to contribute to the neurodegeneration. However, the pathogenic mechanism is not completely understood. To gain deeper insights in the alterations of neuronal and glial cells and underlying pathogenic mechanisms, we recently developed a human *in vitro* cell system based on patient-specific induced pluripotent stem cells (iPSCs).

iPSCs were obtained by retroviral reprogramming using the four transcription factors SOX2, KLF4, OCT4 and cMYC. The iPSCs were differentiated into neural progenitor cells. After neural induction and terminal differentiation, cells were positive for the neuronal markers beta III-Tubulin and MAP2 and also for the glial marker GFAP.

Cells were further analyzed by flow cytometry demonstrating an increased amount of GFAP positive cells in the cell line with NPC1 mutation. In addition western blot analysis revealed an increased amount of GFAP in these cells compared to the healthy control cell line. These results indicate a disease-caused gliosis as described in the NPC1-mouse model so far.

Furthermore, we observed changes in cytoskeleton proteins of the intermediate filament (IF) type III family, in glia cells as well as in neuronal cells. The amount of phosphorylated IFs, determined by western blot using special buffer compositions, was decreased in cells carrying NPC1 mutation. Also immunocytochemical data elucidated changes in the aggregation of IFs, where NPC1-deficient cells displayed an aggregation of longer disorganised bundles of vimentin as well as GFAP. The observed changes might be part of a damage-response mechanism, as discussed for other neurodegenerative diseases like Alzheimer´s disease.

Further studies are aimed to elucidate the underlying mechanism of gliosis and impaired IF cytoskeleton in astrocytes. Especially the impact of disturbed IFs on structural organisation, vesicle transport, as well as neuron-glia interaction, which are discussed to contribute to the pathogenic mechanism in NPC1 disease, will be investigated.

T05-34A

Fumaric acid esters induce hypoxia-induced factor 1α signaling in oligodendrocyte precursor cells

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Amyotrophic lateral sclerosis (ALS) is a fatal neurological disease with still few therapeutic options. Recently, a new drug containing dimethylfumarate (BG00012) which belongs to the group of fumaric acid esters (FAEs) has been approved for treating multiple sclerosis. FAEs can induce the transcription factor Nrf2 which leads to neuroprotection via reducing oxidative stress. In addition FAE induce hypoxia-induced factor 1α (HIF-1α) evoking a pseudohypoxic reaction in glia cells. This might be especially relevant in regard to the lactate shuttle between glia cells and neurons. In ALS a dysfunctional lactate shuttle between oligodendrocytes and neurons might be involved in the pathogenesis. Here we investigate if FAEs induce a psydohypoxic response in oligodendrocyte precursor cells (OPCs) related to the release of lactate. We show that FAEs induce HIF-1alpha genes (VEGF; Glut-1) in the OPC cell line OLN-93 as well as in primary OPC from wild type and transgenic mice expressing mSOD1(G93A), which is a well described mouse model of ALS. Reporter assays demonstrated that the FAE induced VEGF induction was indeed HIF-1α dependent but also Nrf2 dependent. In addition FAE induced the transcription of the lactate transporter MCT-1 and a release of lactate in primary OPCs, suggesting that FAE treatment might indeed stimulate the lactate shuttle between oligodendrocytes and neurons. This might be an additional mechanism involved in the therapeutic effect of dimethylfumarate.

T05-35A

Microglia induce neuroprotective astrocytes via $P2Y_1$ receptor down-regulation

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Activation of microglia and astrocytes occurs in response to various brain injury including traumatic brain injury (TBI). Although they show different spatiotemporal patterns with initial microglial activation and following astrocyte activation, the mechanism how they cooperate and its relation to pathophysiology remained unclear. Here, we report that microglia induce neuroprotective phenotype of astrocytes after brain injury. TBI triggered initial microglial activation in the injury core, followed by astrocyte activation in the peri-injury region to form scar-like structure. The scar size and the number

of dead neurons were positively co-related. When microglial activity was pharmacologically blocked, the scar size increased. To clarify the detailed mechanisms, we used an *in vitro* traumatic injury model. Astrocytes elongated their processes toward the injured area which was dramatically enhanced in the presence of microglia. Microglia-derived inflammatory cytokines and $P2Y_1$ receptor down-regulation in astrocytes were found to mediate in the enhancement. To further confirm pathophysiological roles of astrocytic $P2Y_1$ receptor *in vivo*, we generated two lines of mice in which astrocytic $P2Y_1$ receptors were selectively overexpressed or knocked out. Astrocyte-selective $P2Y_1$ receptor overexpression (astro-$P2Y_1$OE) enlarged the scar size whilst astrocyte-selective $P2Y_1$ receptor knockout (astro-$P2Y_1$KO) diminished in the scar size. In addition, astro-$P2Y_1$OE and astro-$P2Y_1$KO mice showed exacerbated and ameliorated the TBI-induced neuronal damages, respectively. These data suggest that $P2Y_1$ receptor level in astrocytes is necessary and sufficient to control neuroprotective phenotype of astrocytes which is triggered by initially-activated microglia. Taken together, the microglia-astrocyte interaction is essential for formation of neuroprotective astrocytes, for which purinergic signal has an indispensable role.

T05-36A

Study of the CD163 receptor in Parkinson's disease: a prospective biomarker?

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Neuropathological processes in Parkinson's disease (PD) are related to abnormalities of a presynaptic protein called alpha-synuclein (AS), whose aggregation leads to formation of amyloid fibrils in Lewy bodies, which are the hallmarks of PD. CD163 is a scavenger receptor normally expressed on peripheral monocytes/macrophages and whose expression is altered upon inflammation. Shedding of the CD163 from the cell surface occurs in healthy human monocytes and produces a soluble CD163 (sCD163) plasma protein. Interestingly, a positive correlation is seen between the sCD163 plasma level and the severity of various pathologies. Previous studies from our group report an increased infiltration of CD163+ cells and that CD163+ monocyte/macrophages may be used as delivery tool to modify microglia response to neurodegeneration in a neurotoxic rodent PD model. Hence, we hypothesized that there are changes in CD163 in PD and modulation of the inflammatory response can be a valid therapeutic approach in PD. We are analyzing expression of CD163 in postmortem human brain tissues by immunohistochemistry as well as the sCD163 by ELISA to evaluate shedding phenomenon. So far, we have seen an increase in sCD163 in CSF and serum of PD patients when compared to healthy control group and there was a gender effect with females showing higher sCD163 than males. We are currently studying the CD163+ cell population in PD patients as well as the possible influence of AS in the CD163 expression and/or shedding. Our results aim to further evaluate the CD163 population as a therapeutic target in PD as well as to assess the potential value of the sCD163 as a putative biomarker for PD.

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T05-37A

Towards the understanding of the molecular mechanism of vanishing white matter

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Vanishing white matter disease (VWM) is one of the most prevalent genetic childhood white matter disorders. The disease is characterized by ataxia and spasticity. Diagnosis is performed by MRI, which shows progressive white matter degeneration, aggravated by minor head trauma or fever. The lack of

myelin and scar tissue formation in the affected area may result from a maturation defect in astrocytes and oligodendrocytes[1].

VWM is caused by recessive mutations in the eukaryotic initiation factor 2B (eIF2B). This enzyme regulates the protein synthesis rate in every cell type and is regulated by various stressors, including heat shock.

Our project aims to unravel the molecular mechanisms that underlie VWM. Although we have acquired increasing insights into the VWM brain pathology, it remains elusive how a defect in eIF2B causes a disease that almost exclusively affects the white matter. We developed a representative VWM mouse model homozygous for a mutation in eIF2B.

In this study we will determine protein synthesis rates of astrocytes in primary cultures isolated from wt and VWM mouse brains. For this we will use a combination of L-Azidohomoalanine (AHA) and stable isotope labeling with amino acids in cell culture (SILAC)[2]. AHA is used to label newly synthesized protein by the incorporation of AHA instead of the amino acid methionine. AHA-labeled proteins can be separated from the already existing proteins by binding the incorporated AHA to beads. SILAC will be used to discriminate between wt and VWM proteins in the final mass spectrometry analyses.

The cell labeling experiments show that we can label primary mouse astrocytes efficiently with AHA and SILAC. Furthermore we are able to detect 72 proteins that are differentially expressed between WT and VWM astrocytes after a two hour AHA pulse. Currently, we are investigating how modulation of eIF2B activity affects the protein synthesis profile of WT and VWM astrocytes.

The representative VWM mouse model together with the genome-wide assay, pulsed AHA-SILAC labeling, allow us to gain further insight in the molecular mechanism of this disease.

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T05-38A

Soluble epoxide hydrolase inhibition provides multi-target therapeutic effects in rats after spinal cord injury

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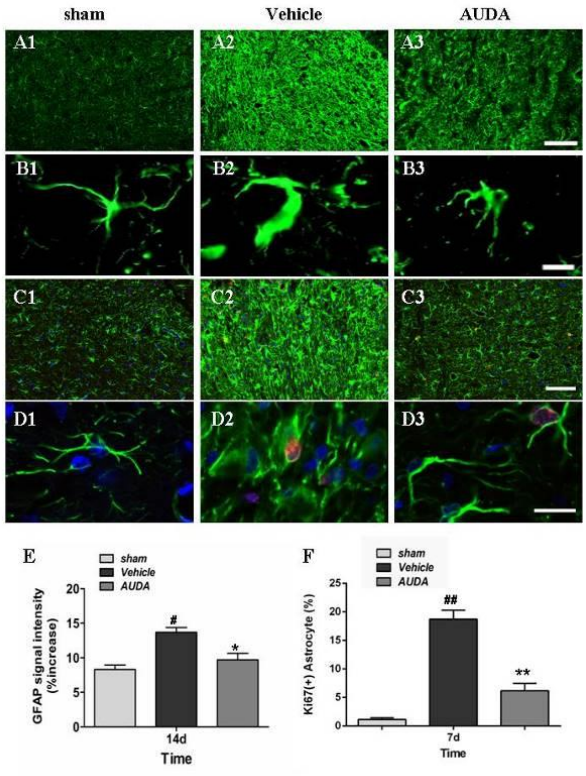
Multiple players are involved in motor and sensory dysfunctions after spinal cord injury (SCI). Therefore, therapeutic approaches targeting these various players in the damage cascade hold considerable promise for the treatment of traumatic spinal cord injury. Soluble epoxide hydrolase (sEH) is an endogenous key enzyme in the metabolic conversion and degradation of P450 eicosanoids called epoxyeicosatrienoic acids (EETs). sEH inhibition has been shown to provide neuroprotective effects upon multiple elements of neurovascular unit under cerebral ischemia. However, its role in the pathological process after SCI remains unclear. In this study, we tested the hypothesis that sEH inhibition may have therapeutic effects in preventing secondary damage in rats after traumatic SCI. sEH was widely expressed in spinal cord tissue, mainly confined to astrocytes, and neurons. Administration of sEH inhibitor AUDA significantly suppressed local inflammatory responses as indicated by the reduced microglia activation and IL - 1 β expression, as well as the decreased infiltration of neutrophils and T lymphocytes. Meanwhile, reactive astrogliosis was remarkably attenuated. Furthermore, treatment of AUDA improvedangiogenesis, inhibited neuron cells

apoptosis, alleviated demyelination and formation of cavity and improved motor recovery. Together, these results provide the first in vivo evidence that sEH inhibition could exert multiple targets protective effects after SCI in rats. sEH may thereby serve as a promising multi-mechanism therapeutic target for the treatment of SCI.

Image



T05-39A

Activation of NO synthase and NO production in crayfish neurons modulates survival and death of satellite glial cells induced by photodynamic impact

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Neuroglial interactions under external impacts may be mediated by nitric oxide (NO) that is produced from L-arginine by neuronal (nNOS) or inducible (iNOS) NO synthase. We have recently demonstrated that NO protected glial cells in the crayfish stretch receptor (CSR), a simplest neuroglial preparation consisting of a single sensory neuron enveloped by satellite glia, from necrosis induced by photodynamic impact (PDT), a strong inducer of oxidative stress and cell death. At the same time, NO enhanced PDT-induced apoptosis of glial cells. In the present work we studied PDT-induced NO production in CSR using 4,5-Diaminofluorescein diacetate (DAF-2DA), a fluorescent probe for NO. We also studied nNOS expression in CSR neurons and glia using nicotinamide adenine dinucleotide phosphate-diaphorase (NADPH-d) histochemistry, a marker for NOS expression. NO production was evaluated as a function of PDT duration, and NOS activation was assessed as a function of the time interval after PDT. Our experiments showed that PDT induced transient increase in NOS activity and NO production and then inhibition of both processes. Cytochemical staining and immuno-fluorescence microscopy showed that NOS was localized mainly in the neuronal perikarion. Surprisingly, we also

observed profound nNOS fluorescence in the neuronal nucleoli. nNOS was also found in the receptor muscles of both sensory neurons. Therefore, NO produced in the crayfish mechanoreceptor neurons differently modulated PDT-induced necrosis and apoptosis of satellite glial cells. This work was supported by grants of RFBR (14-04-00741 and 15-04-05367 A), RSF (14-15-00068) and Russian Ministry of Education and Science (Research organization No 790).

T05-40A

A DAP12-dependent signal promotes pro-Inflammatory polarization in microglia following nerve injury and exacerbates degeneration of injured neurons

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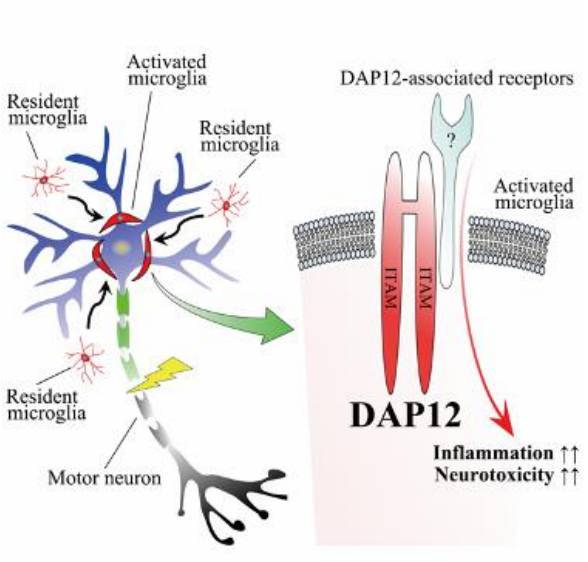
Activated microglia have paradoxical roles such as neurotoxic and neuroprotective functions. Molecules expressing on microglial surfaces are assumed to play crucial roles in determining those microglial characteristics. DNAX activation protein of 12 kDa (DAP12), which is a transmembrane adaptor protein known to regulate activation of osteoclasts and macrophages, is expressed by microglia in central nervous system. Although implications of microglial DAP12 signaling in pathogenesis of neurodegenerative diseases such as Alzheimer's disease, Parkinson's disease and amyotrophic lateral sclerosis (ALS) have been suggested, little is known about DAP12 function in determination of injured motor neuron fate. In this study we have addressed consequences of DAP12 mediated signals in microglia using a mouse hypoglossal nerve injury model. Although initial morphology indicating microglial activation and microglial accumulation in nerve injured hypoglossal nucleus were not significantly different between wild type (WT) and DAP12 deficient (KO) mice, the duration of microglial activation and accumulation after nerve injury was shortened in mice lacking DAP12. Interestingly, expression of M1-phenotype markers including pro-inflammatory cytokines, which were induced in WT, was markedly suppressed in DAP12 KO mice. As the consequence of the alteration of microglial phenotype due to DAP12 deficiency, the survival ratio of nerve injured neurons significantly increased in the DAP12 KO mice. These results suggest that DAP12-mediated microglial activation following axotomy promotes pro-inflammatory responses, and thereby exacerbates neurotoxicity. Therefore, DAP12 could be a potential therapeutic target for the protection of neuronal degeneration caused by microglial activation.

Image



T05-41A

Neurofibrillary degeneration upregulated Hsp27 expression in astrocytes in transgenic rat brain

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It has been shown that Hsp27 played important role in Alzheimer's disease. The elevated levels of Hsp27 in the cortex of brains from AD patients were demonstrated in several independent studies. In the present study we utilized transgenic rat model expressing human truncated tau that developed extensive neurofibrillary pathology. We found that Hsp 27 is mainly expressed in reactive astrocytes. Hsp27-positive astrocytes were detected in close proximity to neurofibrillary tangles. Stereological quantification demonstrated significant increase in the number of GFAP-positive (reactive) astrocytes in TG brains compared to WT animals (1.7-fold increase; $p<0.002$). Further, we observed a selective increase of Hsp27-positive reactive astrocytes in transgenic rat brains (16-fold increase; $p<0.01$). Interestingly, more than 30 % of the GFAP-positive astrocytes were also immunoreactive to Hsp27 in transgenic rat brain, whereas in control animals only 3 % of astrocytes were immunoreactive to both markers ($p<0.05$). Confocal study supported our immunohistochemical data showing that significant part of GFAP positive astrocytes displayed increased expression of Hsp27 in TG animals. Finally, we found that Hsp27 positive reactive astroglial cells were localized in a close proximity of AT8 positive neurofibrillary tangles and AT8 positive dystrophic neurons. To sum up, we found that Hsp27 is an important marker of activated astrocytes in neurodegenerative environment. We suggest that Hsp27 may be involved in the tau neurodegenerative cascade.

T05-42A

Understanding ApoD neuroprotective function: ApoD distribution in pH-dependent subdomains of the astroglial lysosomal compartment upon metabolic and oxidative stress

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Apolipoprotein D (ApoD) is expressed in the nervous system, increases with aging and neurodegeneration, and is induced in response to serum deprivation or oxidative stress. To understand how ApoD distribution in different subcellular compartments affects glial cell protecting mechanisms, we analyze the time course of ApoD subcellular traffic upon exposure to low-serum and paraquat stimuli in a human astroglioma cell line.

Confocal microscopy analysis of ApoD localization indicates that secretion is followed by interaction with the plasma membrane, endocytosis via both the caveolin and clathrin-mediated pathways, and location in endosomes. No ApoD is immunodetected in mitochondria, peroxisomes or nuclei.

Interestingly, an important fraction of ApoD is targeted to lysosomes. Large LAMP2 and ApoD-positive organelles indicate ApoD presence in autophagolysosomes as well, which is confirmed by co-localization with LC-3. ApoD presence inside lysosomes and autophagolysosomes is stable over time, suggesting an active role in lysosomal-autophagy function.

We have developed a method to analyze ApoD distribution differences in lysosomes after *in vivo* pH measurement of individual lysosomes, and have found that metabolic or oxidative stress treatments change not only lysosomal pH, but also the ApoD distribution pattern within lysosomal populations. This close relationship between lysosomal pH and distribution of ApoD is currently being analyzed under conditions of pharmacological induction or inhibition of autophagy, bringing light into the role of this classically extracellular apolipoprotein in lysosomal performance.

The interaction and traffic of ApoD inside neurons, once secreted by glia, is also being studied and analyzed in the context of the neuronal protection role already reported for ApoD.

In the light of these new findings, previous hypotheses on ApoD function in glial cells must be refined: the control of plasma membrane and lysosome/autophagosome functions must be key elements in the neuroprotective role of this lipid-binding protein.

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T05-01B

A_{2A} receptor blockade prevents microglia reactivity triggered by elevated hydrostatic pressure

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Glaucoma is a retinal degenerative disease characterized by degeneration of retinal ganglion cells (RGCs) and damage of the optic nerve. One of the major risk factors for the development of glaucoma is the increase in intraocular pressure (IOP). Several reports have shown that early in disease microglia become reactive which may contribute to RGC loss.

Adenosine is a neuromodulator in the central nervous system acting through the activation of four receptors, A_1, A_{2A}, A_{2B}, A_3. In the brain, the blockade of $A_{2A}R$ prevents microglia reactivity and confers protection in several models of neurodegenerative disorders.

In this study, we aimed to assess the effects of elevated hydrostatic pressure (EHP) in the reactivity of microglia and to investigate the potential protective properties of $A_{2A}R$ blockade.

Retinal primary neural cell cultures and microglial cell cultures (BV-2 cell line) were pre-treated with 50 nM SCH 58261, a selective $A_{2A}R$ antagonist, and exposed to EHP (70 mmHg above normal atmospheric pressure), to mimic increased IOP. Control cells were incubated in a standard cell incubator (normal atmospheric pressure; 760 mmHg). Microglial cell morphology, phagocytosis, proliferation, migration, inflammatory mediators, and cell death were assessed.

Exposure of retinal neural cell cultures to EHP increased cell death and the number of microglia with engulfed dead cells. Also, EHP increased microglia reactivity and inflammatory response in retinal mixed cultures, assessed by cell morphology and ELISA. Furthermore, total cell proliferation and microglia proliferation increased upon exposure to EHP. Exposure of BV-2 cells to EHP increased $A_{2A}R$ protein levels, microglia migration, phagocytosis and inflammatory response. Pre-treatment with $A_{2A}R$ antagonist prevented cell death, changes in microglia morphology, proliferation, phagocytosis and migration induced by EHP.

In summary, our results show that EHP triggers microglia reactivity. The blockade of $A_{2A}R$ reduces microglia activation and retinal cell death elicited by EHP, suggesting that $A_{2A}R$ antagonists could have beneficial effects for the treatment of glaucoma.

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T05-02B

Neuroprotective effects of the nucleoside guanosine under acute hyperammonemia in a rat model of hepatic encephalopathy

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Hepatic Encephalopathy (HE) is a serious complication of both acute and chronic liver failure, being one of the main causes of death in patients with impaired liver function. Although the physiopathology of HE is not yet fully understood, hyperammonemia and astrocyte dysfunction seem to be the main agents, since high concentrations of ammonia can activate inotropic glutamate receptors (NMDA) and increase extracellular glutamate levels. It is known that guanosine (GUO) has a neuroprotective effect by inducing an increase in the uptake of glutamate by astrocytes when this neurotransmitter is high. The objective of the presenting study was to investigate the effects of the nucleoside GUO on clinical, neurochemical and electrophysiological parameters in a model of acute ammonia intoxication induced by intraperitoneal (i.p.) injection of ammonium acetate. We used 90 days male Wistar rats, which received two i.p injections: the first containing control or GUO 60mg/kg solution, and the second, 20 minutes later, containing either vehicle or 550 mg/kg of ammonium acetate. Animals were then observed during 50 minutes and classified based on a neurological scale divided into three stages: normal, coma (absence of corneal reflex) and death. On a second cohort of rats, we implanted surface brain electrodes one week prior to the experiment. We evaluated the severity of encephalopathy by electroencephalogram (EEG) spectral analysis during 75 minutes after the second injection. In a third cohort of rats, we evaluated the cerebrospinal fluid (CSF) for ammonia and amino-acid levels, glutamate uptake on brain slices and glutamine synthetase activity and oxidative stress on cortex, using time mark of 10 and 20 minutes after the second injection. As results, we found that GUO administration did not change the proportion of animals that developed to coma, but it reduced the time spent in a comatose state and drastically reduced the mortality. The EEG analysis corroborates the clinical data, showing that GUO improved the neurological status when compared to control rats. The control group showed reduced glutamate uptake by astrocytes, while the group treated with GUO showed normal uptake levels. Moreover, the animals treated with GUO presented improvement of CSF parameters and better oxidative stress levels. Concluding, this study may provide new evidence of the the neuroprotective effect of GUO and the mechanism of action of purine derivatives of guanine by potentially modulating the glutamatergic system under glutamatergic excitotoxicity situations.

T05-03B

EAE is associated with increased expression of mitochondrial proteins within the dorsal spinal cord: implications for pain in the disease

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Multiple sclerosis (MS) is an inflammatory demyelinating disease of the central nervous system (CNS) that leads to severe neurological disabilities. In addition impairing motor function, MS is also associated with a high incidence of neuropathic pain. Previously our lab has shown that the animal model experimental autoimmune encephalomyelitis (EAE) displays neuropathic pain behaviours before and at the onset of clinical signs. Pain hypersensitivity in EAE has been associated with significant changes in glial reactivity within the lumbar dorsal horn of the spinal cord; an area critical for the processing of pain related signals. We have recently observed that an exercise regimen in mice with EAE can diminish pain hypersensitivity in the model. We have found that EAE mice exhibiting reduced pain behaviours also have decreased signs of oxidative stress in the spinal cord. To further characterize this, we have now examined mitochondrial protein levels within the dorsal spinal cord at the onset of clinical signs in EAE. We find an increase in the levels of both the outer mitochondrial membrane protein Tom20 and the complex IV protein (COX IV) compared to control mice. Using immunohistochemistry we have also observed that the significant elevations in the levels of Tom20 and COX IV are localized in the dorsal horn and white matter lesions associated with the disease. Within these lesions, Tom20 predominately co-localizes with the macrophage/microglial marker Iba-1 and GFAP positive astrocytes. In the superficial dorsal horn, Tom20 is also found in Iba-1 and GFAP positive cells. However, we find an additional increase in the neuronal expression of Tom20 in this region. These results indicate that mitochondrial changes may have a role in EAE associated hypersensitivity. Further research is being conducted to determine whether EAE pain hypersensitivity can be improved by the stabilization of mitochondrial function.

T05-04B

Blockade of adenosine A_{2A} receptor confers neuroprotection against retinal ischemia-reperfusion injury through the control of neuroinflammation

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Neuroinflammation mediated by microglial cells has been associated with the development and progression of retinal disorders. Retinal ischemia-reperfusion (I-R) injury contributes to several ocular diseases including glaucoma, which is characterized by degeneration of retinal ganglion cells (RGCs) and is accompanied by a neuroinflammatory response involving retinal microglial cells. Moreover, it was demonstrated that I-R injury induces neuronal damage, and triggered microglia activation increasing retinal neuroinflammation

Increasing evidence has demonstrated that blocking adenosine A_{2A} receptor ($A_{2A}R$) prevents neurodegeneration by modulating the release of noxious factors by activated microglia. The aim of this work was to evaluate whether $A_{2A}R$ blockade could prevent microglial reactivity and retinal cell death induced by ischemia-reperfusion (I-R) injury.

Retinal ischemia was induced in one eye by elevating the intraocular pressure (IOP) for 60 min, which was followed by 24 h of reperfusion. The contralateral eye served as the control eye. The $A_{2A}R$ antagonist was administrated by intravitreal injection (SCH 58261; 100 nM, 5 µl) prior to I-R.

Retinal microglial reactivity induced by I-R injury was prevented by administration of SCH 58261. The $A_{2A}R$ antagonist inhibited the increase triggered by I-R injury in the expression and levels of inflammatory cytokines IL-1β and TNF, as well as GFAP expression. The increased number of retinal cell death by apoptosis induced by I-R was significantly decreased in retinas treated with $A_{2A}R$ antagonist. Additionally, blockade of $A_{2A}R$ prevented the I-R induced retinal ganglion cell loss, an effect that was also observed with neutralization of TNF and IL-1β.

These results suggest that the neuroprotective effect of $A_{2A}R$ antagonist, associated with its ability to control retinal microglial cell reactivity, can be used as potential therapeutic targets for glaucoma, or other retinal diseases involving neuroinflammation.

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T05-05B

Inhibition of Casein Kinase 2 reduces AMPA-induced oligodendrocyte death through JNK signaling and ER stress regulation

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Casein kinase 2 (CK2) is a ubiquitous serine-threonine kinase that plays a critical role in the regulation of cell proliferation, survival and cell death, and its activity has been found to be highly enhanced in some human and experimental diseases. CK2 mediates survival or apoptosis via AKT and JNK pathways, respectively. Here we have analyzed the involvement of CK2 in AMPA-induced excitotoxicity using rat cultured oligodendroglial cells. First, we observed that brief and moderate AMPA receptor activation in oligodendrocytes triggered CK2 activity which was abolished by CK2-specific inhibitor TBB. We also found that the CK2 inhibitors TBB, DRB and resorfurin diminished

mitochondrial dysfunction and subsequent cell death provoked by AMPA. In addition, moderate excitotoxic insults caused an early and potent activationof both the p-JNK/c-Jun pathway and the pro-apoptotic BH3-only proteins, events that were significantly attenuated in the presence of CK2 inhibitors. Regulation of this pro-apoptotic signaling may explain, at least in part, the protective effects that CK2 inhibitors exert on AMPA-mediated damage in oligodendrocytes.

In turn, CK2 and some of its substrates are located at the endoplasmic reticulum (ER) where CK2 can phosphorylate, regulate and modify the role of ER resident proteins in response to stress. However, it is unclear whether the function of CK2 on ER stress is or not protective. AMPA exposure triggers ER stress in oligodendrocytes and, based on this knowledge, we investigated a possible linkage between CK2 activity and ER stress using cultured oligodendrocytes. Indeed, we found that CK2 is expressed at the ER of oligodendrocytes. In addition, CK2-inhibitor TBB decreased the expression levels of chaperon protein GRP78/94, a molecular ER stress sensor, and attenuated AMPA-induced ER stress via reducing eIF2a phosphorylation.

Together, these results assess the involvement of CK2 in oligodendrocyte demise. Specifically, CK2 plays an important role in AMPA-mediated oligodendrocyte death through its interaction with JNK-dependent signaling and ER stress mechanisms. Therefore, pharmacological modulation of this kinase may help in the development of novel drugs with therapeutic potential for demyelinating diseases.

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T05-06B

Intravital microglial lysosome imaging

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The study of microglial lysosomes in vivo in brain tissue is limited, among other factors, mainly due to lack of selective marker delivery to the brain. The blood brain barrier (BBB) selectively allows or excludes molecular transport in and out of the brain. Dextrans conjugated with dyes are suitable markers to be used in the study of uptake and internal processing of exogenous materials by phagocytic and endocytic pathways, thus making them suitable as long-term tracers. In culture, microglia take up anionic dextran molecules selectively as compared to other cell types, thereby allowing for the study of lysosomal pH and trafficking as well as endocytic pathways. However, dextran delivery to the brain is limited due to their low BBB permeability. To study microglial lysosomes in vivo we developed a method for delivery of dextran molecules directly into the cerebrospinal fluid by intrathecal spinal cord injection. Intrathecal injection of dextrans, conjugated with FITC and Rhodamine fluorophores, was performed in C57Bl/6 mice followed by intravital imaging and immunohistochemistry at various time points after injection. Intravital multiphoton microscopy imaging of dextran delivery to mouse cortex was performed through a cranial window. Additionally, in order to identify microglia and determine whether cells had been loaded with dextran, brain tissues were thereafter sectioned and stained again an Iba1 antibody. Immunofluorescence showed selective internalization of dextrans by lysosomes in Iba1-positive cells (by FITC-Iba1 spatial correlation) in cerebellum and cortical layer III 10 days after the last intrathecal injection. Moreover intravital imaging showed dextran dyes within lysosomes in microglia-like cells in the mouse cortex. These results provide a valuable methodology to be used for in vivo study of microglial lysosomes and provide new information regarding compound delivery to the brain relevant for drug discovery.

T05-07B

Knocking out the Na^+/Ca^{2+} Exchanger NCX3 impairs oligodendrocyte lineage responses, anticipates the onset, and increases the severity of Experimental Autoimmune Encephalomyelitis

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The Na^+/Ca^{2+} exchanger NCX3, recently identified as a myelin membrane component, is involved in regulating intracellular Ca^{2+} concentration during oligodendrocyte maturation. The importance of this key mediator of sodium and calcium homeostasis was examined in myelin oligodendrocyte glycoprotein (MOG)-induced experimental autoimmune encephalomyelitis (EAE), an animal model of Multiple Sclerosis. Western blotting and quantitative colocalization studies performed in wild-type *ncx3+/+* mice at different stages of EAE disease showed that NCX3 protein was intensely upregulated at chronic stage where it was intensely coexpressed by the OPC marker NG2 and the premyelinating marker CNPase. Homozygous mice lacking *ncx3* gene (*ncx3-/-*) not only displayed a reduced diameter of axons and intact myelin ring number but also a dramatic decrease in OPC and pre-myelinating cells in the white matter spinal cord if compared to congenic *ncx3+/+* mice at chronic disease stage. Accordingly, homozygous *ncx3 -/-* and heterozygous *ncx3 +/-* mutants displayed an anticipated development of EAE disease and increased severity of clinical symptoms. Interestingly, in *ncx3-/-* mice, the number of immune T-cell subsets, revealed by cytometry analysis at the peak of EAE disease, was not statistically different from those measured in congenic *ncx3+/+*. Taken together our findings demonstrate that knocking out NCX3 exchanger impairs oligodendrocyte lineage responses and worsens clinical symptoms in EAE-induced Multiple Sclerosis without involving alterations in immune T-cell population.

T05-08B

A role of SRY on gender-selective modulation of astrocytic cell viability by oxidative stress

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Question: Gender differences across biomedical research have recently emerged as an issue in many pathological conditions. Additionally, neurological disorders including autism, Alzheimer's disease and Parkinson's disease have gender difference of incidence rate and symptom severity. It is well known that male and female neurons respond differently to various stimuli due to a combination of genetic and hormonal dependency. But gender-related astrocytic responses to some stimuli are less well defined yet. In this study, we investigated the effect of sex-determining region Y (SRY), Y-chromosome limited expression gene, on cell death susceptibility in oxidative stress-induced rat primary astrocytes.

Methods: Rat primary astrocytes cultures were prepared from postnatal day 1 male or female pups. Cellular viability and SRY expression were identified in hydrogen peroxide (H_2O_2) stimulated condition. Knockdown of SRY was employed by SRY siRNA transfection in rat male astrocytes.

Results: Male astrocytes are more susceptible to stress from reactive oxygen species compared to female astrocytes. Interestingly, protein and mRNA expressions of SRY were increased in H_2O_2-stimulated male astrocytes. In chemical inhibitor study, H_2O_2-induced SRY expression is inhibited by Erk and NF-κB inhibitors, concomitant with the rescue of cellular viability suggesting the role of SRY on the modulation of astrocytes cell survival. Conclusively, reduced male astrocyte viability by oxidative stress as well as intracellular antioxidant level was modulated by SRY siRNA transfection.

Conclusions: We concluded that SRY modulates cellular viability and anti-oxidant defense mechanism of astrocytes possibly via Erk and NF-κB pathway in oxidative stress-induced rat primary

astrocytes. These results imply that SRY is a candidate genetic factor underlying gender difference in glial responsiveness to neurotoxic stimuli.

T05-09B

Neuroprotective effects of guanosine in an glutamatergic excitotoxic condition in hippocampal slices from adult mice

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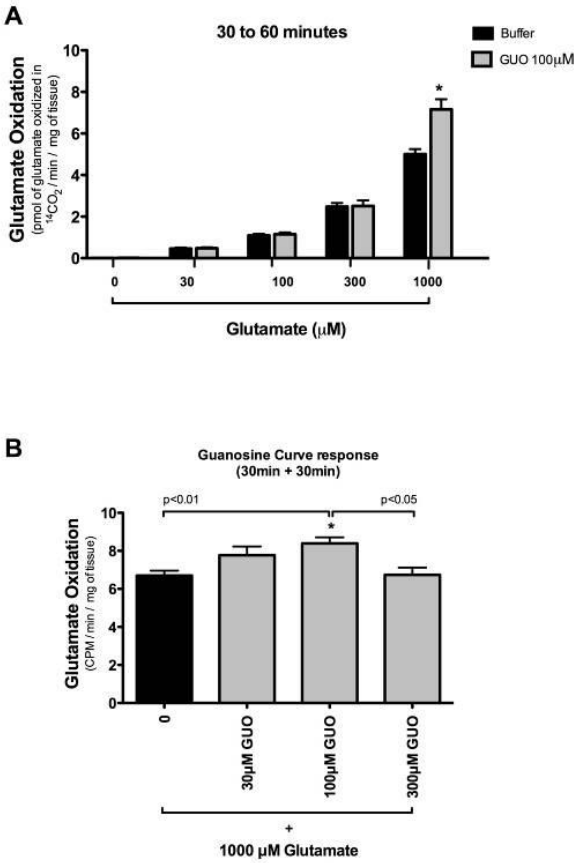
The glutamatergic excitotoxicity is involved in various pathological processes in the brain. In this context, astrocytes play an important role in glutamatergic system metabolizing and capturing glutamate (Glu). One way that these astrocytes can use this Glu through the TCA cycle to produce ATP. In addition, excess of glutamate in the synaptic cleft increases neuronal excitability. The guanosine (GUO) acts as neuroprotective substance, however the mechanism of this action is unclear. Our group has several studies showing that GUO increased astrocytic Glu uptake and antioxidant content. The objective of this study was to investigate the mechanism by which GUO exert neuroprotection in mice hippocampal slices incubated with excitotoxic Glu concentration. The mice were sacrificed by decapitation and the hippocampi was removed, weighed and sliced in a chopper equipment (McIlwain) to 300um. These slices were preincubated at 4 ° C and then washed with media containing artificial cerebrospinal fluid (aCSF) containing glucose (5 mM). After this process, the slices are incubated with different concentrations of Glu and GUO in different times in the metabolic bath with constant shaking at 37 °C and aerated with a gas mixture (95% CO2: 5% O2), to observe the better neuroprotective effect of GUO. First, we analyzed the concentration of Glu that produce an increase in Glu utilization to CO_2 (L-[14C(U)]-Glu) without cell injury; we proceeded with a curve using different concentrations of Glu (0, 30, 100, 300 and 1000uM) and different times (0 - 2h). We observed that the concentration of 1000uM of Glu increase Glu oxidation in the time between 30-60 minutes of incubation without cell injury (measured by MTT and flow cytometry). In this condition (Glu concentration and time), we make a curve of GUO (0 at 300uM) and showed that 100uM of GUO increase (35%) Glu oxidation. Behind this result, we study the effect of 100uM of GUO on Glu uptake (L-[3,4-3H]-glutamate) in mice hippocampal slices incubated with 1000uM of Glu per 1h (30-60min) and in this situation GUO increase Glu uptake (around 45%). This is the first time a study shows that GUO promotes an increase in Glu uptake and utilization together. However, more studies are necessary to determine if GUO act intracellularly or directly in membranes.

Image

Figure 1. Curves



T05-10B

Galactosylceramidase (GALC) enzymatic activity and psychosine accumulation in central and peripheral nervous system cells and tissues from wild-type and Twitcher mice

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Globoid cell leukodystrophy (GLD, or Krabbe disease) is an autosomal recessive, neurodegenerative rare disease caused by deficiency of the lysosomal enzyme galactocerebrosidase (GALC).

GALC degrades galactosylceramide, a major component of myelin, and other terminal b-galactose-containing sphingolipids, including the cytotoxic D-galactosylsphingosine (psychosine, PSY). GALC loss of function causes increased PSY levels in the neural tissues, leading to widespread degeneration of oligodendrocytes and Schwann cells and subsequent demyelination.

Still little is known about the molecular mechanisms by which PSY imparts toxicity and no cure is currently available for GLD [Graziano et al., Gene 2015; Voccoli et al., Cell Death Dis 2014]. Unfortunately, the systemic GALC administration is not effective because of the presence of the blood

brain barrier (BBB). The development of an enzyme replacement therapy (ERT) that takes advantage from the recent nanomedicine advancements to cross BBB would be one of the most promising options for curing GLD.

In order to help in developing ERTs in the mouse GLD model, the Twitcher mouse (TWI), a study of GALC activity, PSY levels and viability in fibroblasts and different central and peripheral nervous system (CNS and PNS) cell types and tissues is here presented.

We have set up cultures of primary wild-type (WT) and TWI fibroblasts and cells from CNS (i.e. astrocytes, oligodendrocytes and neurons) and characterized their GALC expression and activity by western-blotting and fluorimetric methods. These measurements were carried out both in cells lysates and in culture supernatants, allowing us to assess the level of enzyme exocytosis in different cell types. An analogous analysis is also reported for CNS and PNS tissues, such as: brain, optic and sciatic nerves.

PSY quantification in WT and TWI homogenized brains, optic and sciatic nerves, and in different CNS cell types was performed by an LC-ESI-tandem-MS method. PSY accumulation in tissues was evaluated for TWI mice of different age.

Moreover, data on cell viability of WT and TWI cells treated with different PSY concentrations are presented. Possible effect of autophagy modulators on cell viability will be finally discussed. A disturbance of autophagic pathways was indeed reported for many lysosomal storage disorders [Settembre et al., Autophagy 2008], and it has been linked to a deficiency of trafficking/processing of recombinant therapeutic enzymes, suggesting that autophagy dysfunction may have an impact in determining the efficacy of ERT.

Image

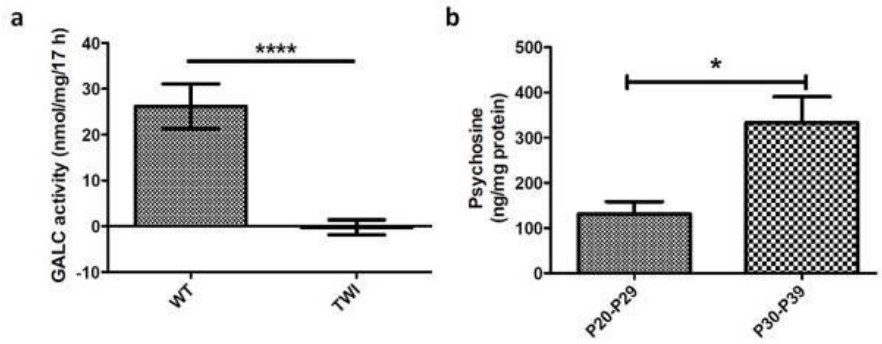

Figure 1 GALC Enzymatic activity in TWI and WT fibroblasts and PSY quantification in TWI brains. (**a**) Quantification of GALC enzymatic activity obtained measuring fibroblast protein extracts from 12 animals (n=6 WT and n=6 TWI mice). Data are reported as mean±SEM; WT vs TWI t-test, P<0.0001. (**b**) PSY content was measured in 19 TWI brains between P20 (time of disease onset; P20-29, n=7) and P39 (disease late stage; P30-P39, n=12). PSY was not detectable in WT animals. Data are reported as mean±SEM; P20-29 vs P30-39 t-test, P<0.05.

T05-11B

Anti-Il34 treatment reduces microglia density

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As the resident immune cells of the brain, microglia are implicated in the pathophysiology of a variety of neurodegenerative diseases, however the potential role(s) are ill-defined. One approach to study the function of microglia is to deplete the cells and assess the effect on disease pathology. We set out to develop a method to deplete microglia by inhibiting the signaling through the CSF1R pathway,

which is required by microglia for survival. Its been shown that inhibition of CSF1R signaling with small molecule inhibitors can deplete microglia in the brain, but the use of these compounds runs the risk of off target effects via insufficient selectivity or non-specific binding. Based on this concern, we took a novel approach and targeted Il-34, one of the two ligands of CSF1R. Genetic knockout of Il-34 has been shown to result in ~ 40 to 60% reduction in microglia density in the cortex. Using an anti-Il34 neutralizing antibody, we were able to reduce microglia density in the cortex by ~40%. Depletion was dose dependent, and is saturable at concentrations that are consistent with the antibody inhibiting Il-34 within the central nervous system. Microglia depletion is also seen in the spinal cord. By morphometric analysis we find a small increase in microglia size. Interestingly, we do not see evidence that those remaining microglia have become activated, nor do we see any evidence of astrogliosis in response to microglia depletion. In ongoing studies we are characterizing the response of microglia signaling to anti-Il34 treatment, which will allow us to apply this method to disease models to assess microglia function related to pathology.

T05-12B

Glial cell-dysfunction and therapeutic potential of trehalose in an early Huntington's disease cellular model

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Huntington's disease (HD) is an autosomal neurodegenerative disease caused by an abnormal expansion of polyglutamines in the protein named huntingtin (Htt). A pathological hallmark of HD pathology is the aggregation of mutant Htt of striatal, cortical neurons and glia. This accumulation causes the selective loss of striatal medium sized spiny neurons in the brain. Up to the present, the role of glia in HD it is poorly understood, little is known and classically has been considered secondary to neuronal disorder but some studies have documented a critical role of glial in the neuronal function. Recent research indicates that astrocytes and microglial cells participate actively in the modulation and transmission of nerve signals, production and release of neurotrophic factors and play crucial roles in adult CNS homeostasis regulation.

In the present study, we analysed at an early development stage the abnormalities observed in R6/1 mice postnatal striatal glial cell cultures, under baseline and stressing conditions using epoxomicin, and the effects of trehalose, a chemical inducer of autophagy with protective properties and chemical chaperone activity, on the pathological glial changes involved in HD.

Detection of glial markers by inmunocytochemical analysis showed that the number of glial progenitors ($A_2B_5^+$) and astrocytes ($GFAP^+$) were significantly diminished in HD cultures respectively (Figure 1A and 1C). In contrast we founded that microglial cells, as isolectin B4+ cells, showed a substantial increase in the HD cultures. In addition the cell body of microglia HD was transformed from highly ramified to an amoeboid state characteristic of activated microglia, even more with epoxomicin treatment. However, trehalose addition compensates the microglia altered morphology improving even their pathological basal state and also reduces the Htt accumulation seen in these cells.

Our results suggest that glia suffers functional changes that can contribute to the neurodegeneration in HD and that trehalose, due to its autophagy activation capacity, anti-aggregation properties, anti-oxidative effects and lack of toxicity, could be very promising for the treatment of HD and for other polyglutamine expansion diseases.

T05-13B

Schwann cells regulate synaptic function at developing neuromuscular synapses

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In our previous studies, we found that embryonic motor neuron (MN) degeneration caused by congenital deficiency of Schwann cells was completely reversed by blocking peripheral neural activity. In order to determine how activity promotes MN death in this paradigm, we characterized synaptic transmission at nascent neuromuscular junctions (NMJ) in Schwann cell-deficient erbB3 mutant mice. Using muscle-expressing GCaMP3 mice and intracellular recording in the presence of a novel myosin-blocking drug, we were able to demonstrate nerve-induced contraction of diaphragm muscle as early as embryonic day 14.0 (E14). In low calcium buffers, we could elicit synaptic depression caused by high-frequency nerve stimulation at these early ages. Using Schwann cell-expressing GCaMP3 mice, we observed Schwann cell activation in response to high- but not low- frequency nerve stimulation, suggesting that Schwann cells respond to neural activity at developing NMJs, similar to the adult. Bath application of muscarine also induced Schwann cell calcium responses, suggesting that acetylcholine (ACh) released by MNs in response to high-frequency stimulation activates Schwann cell-expressing muscarinic ACh receptors at the NMJ. Finally, we characterized NMJs immunohistochemically, ultrastructurally and electrophysiologically in erbB3 mutant mice before the onset of MN degeneration. We found prematurely differentiated synapses, shortened miniature endplate potentials (mEPPS), and delayed onset of synaptic depression in E14.25 erbB3 mutants. Thus, Schwann cells at the developing NMJ regulate synaptic function as well as synaptic maintenance. We propose that embryonic Schwann cells maintain the NMJ (and thus the MN itself) by regulating neuromuscular synaptic activity.

T05-14B

Erythropoietin affects the dynamic brain edema response following experimental traumatic brain injury

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Traumatic brain injury (TBI) and traumatic brain edema is a major contributor to mortality and morbidity. Changes in the function of the blood brain barrier (BBB) and variations in aquaporin (AQP) expression have been proposed to be involved in development of traumatic brain edema, however there is limited data regarding temporal changes of AQP4 and BBB in trauma models. Adult Sprague-Dawley rats were subjected to blunt controlled head trauma (TBI) and followed for up to four days by MRI, immunohistofluorescence, immunohistochemistry and quantitative protein analysis. Non-traumatized animals served as controls. TBI resulted in a midline shift of 8% and a decrease in ADC by 12% at day 1 after injury, indicating a hemispheric enlargement due to cytotoxic edema. The changes persisted at day 4 after injury. The tight junction protein ZO-1 was decreased by 25% and associated with a 20% increased IgG permeability in the perilesional brain parenchyma, indicating BBB dysfunction. AQP4 protein amount was decreased by 20% at day 1. The disruption of the BBB integrity lasted for at least 4 days while the impact on AQP4 levels disappeared between day 1 and 4 post injury. Rats exposed to TBI were then administered EPO 5000 IU/kg i.p. within 30 minutes after trauma and once daily for 4 days. Control TBI animals received saline. The midline shift was not affected by EPO treatment. EPO significantly attenuated the ADC decrease at day 1 after TBI, suggesting that the cytotoxic edema component was reduced by EPO. Furthermore, EPO significantly decreased IgG leakage at day 4 post TBI. Our results indicate that blunt focal brain injury results in a temporal development of brain edema involving both cytotoxic and vasogenic components, a persistent BBB breakdown and a temporary decrease in AQP4. We speculate that the observed decrease in AQP4 may inhibit edema resolution. Further the data suggest that posttraumatic administration of EPO may decrease early phase cytotoxic edema as well as the permeability of the BBB in the later phase of edema development. The dynamic and termporal response to TBI suggests that both types of edema should be targeted in traumatic brain edema treatment.

T05-15B

Iron loading with ferrocene induces iron mismanagement in organotypic hippocampal slices

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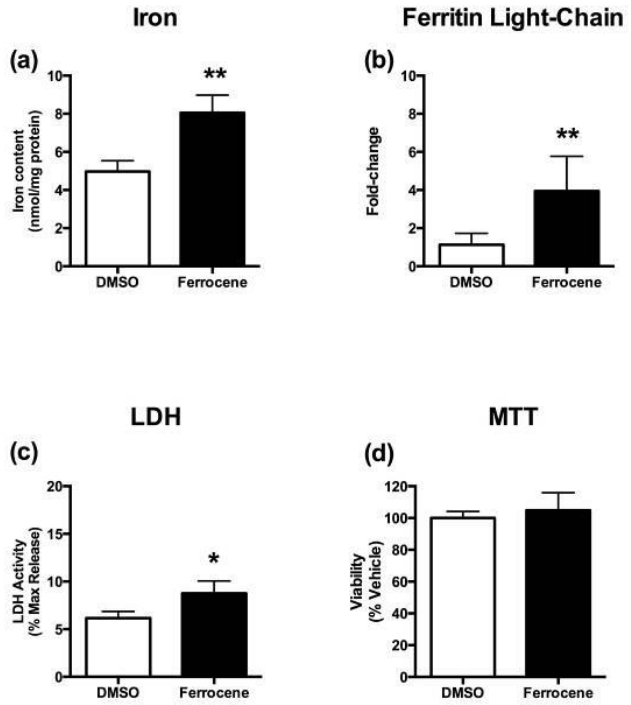
Introduction: Iron may contribute to the pathogenesis and progression of multiple sclerosis and other neurodegenerative diseases. Recent studies have shown alterations in iron distribution and the expression of iron-related molecules. A clearer picture of this metabolic dyshomeostasis of iron might be described using an *ex vivo* slice culture platform. In the present study, we examined the molecules involved in aberrant iron homeostasis by iron-loading organotypic hippocampal slice cultures.

Methods: We generated slice cultures from P10-11 Sprague-Dawley rats and used ferrous ammonium sulfate, ferric citrate and ferrocene as iron-loading reagents at a concentration of 10 uM for 12 hr. Iron levels were quantified using a ferrozine colorimetric assay and viability was assessed using LDH and MTT assays. Using real-time PCR, the expression level of iron-storage molecule ferritin light chain was determined. Cell-specific iron accumulation was then assessed by dual immunofluorescent labelling of ferritin-light chain together with GFAP, OX-42, Olig-2 and NeuN, respectively.

Results: Firstly, we demonstrated a normal iron content in these slice cultures after 10 days in culture (5.61±0.66 nmol/mg) that compared well with age-matched tissue samples (5.99±1.03; $p>0.05$). Moreover, iron content was on a par with values reported for primary cultures of CNS cells. Secondly, we demonstrated differential iron uptake and toxicity after 12 hr exposure to 10 μM (a supraphysiological concentration) ferrous ammonium sulfate, ferric citrate or ferrocene. Thirdly, we showed that 1 μM ferrocene causes maximal iron loading in hippocampal slices. This concentration of ferrocene produced a 1.6-fold increase in iron content (4.97±0.57 to 8.05±0.98 nmol/mg; $p<0.05$) with minimal impact on culture viability. This increase is in line with total iron increases reported in aged and/or diseased post-mortem brain. Furthermore, iron treatment led to significantly higher levels of ferritin light-chain (an iron storage molecule) transcripts ($p<0.01$) and expression of ferritin protein was characterised by immunohistochemistry.

Conclusions: Levels of iron in our neonatal hippocampal slice cultures replicate those measured age-matched samples of hippocampal tissue. Following comparison of different iron reagents, 1 μM ferrocene was found to cause the highest accumulation of iron. This work represents the first reported accumulation of iron in organotypic slice cultures. We believe this model to be a promising platform for studying iron regulation in the CNS.

Image



T05-16B

Biochemical and pharmacological evidence for the existence of spare glutamate transporters - the concept of transporter reserve

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Question: The efficient uptake of synaptic glutamate through glutamate transporters is essential to limit the duration and intensity of excitatory neurotransmission. Altered glutamate uptake by astrocytes may lead to excitotoxic insults and the loss of glutamate transporters has been proposed as one of the cellular mechanisms supporting the development or progression of neurodegenerative diseases, including amyotrophic lateral sclerosis, Parkinson disease and Alzheimer disease. The activity of high affinity glial glutamate transporters can be monitored in cell cultures or tissue preparations by measuring the uptake of radiolabeled substrates. Analyses of experimental data using standard equations of pharmacodynamics allow to calculate kinetic constants and maximal uptake velocity.

Methods: We have developed a model of transfected HEK cells in which the expression of the high affinity glutamate transporter GLT-1 can be manipulated thanks to a doxycycline-inducible promoter. Combining Western blot detection of GLT-1 and substrate uptake studies allowed us to examine the correlation between transporter expression and function.

Results: Our data showed that beyond a substantial level of induction, increasing in the concentration of doxycycline efficiently promoted enhanced expression, but failed to increase the maximal uptake velocity. Detection of cell surface transporter expression using a protein biotinylation approach confirmed the efficient addressing of transporters to the cell membrane. With analogy to the pharmacological concept of receptor reserve, this suggests the existence of transporter reserve or spare transporters. The concept was further validated using the selective GLT-1 blocker WAY-213613. Thus, the sigmoidal curve depicting the inhibition of uptake by increasing concentrations of WAY-213613 was progressively shifted to the right when tested in cells where the transporter density was robustly induced.

Conclusions: As widely documented in the context of cell surface receptors, the existence of spare glutamate transporters could impact on the consequence of physiological or pathological regulation of transporters. Also, tissues where the proportion of spare glutamate transporters is substantial may show some resistance against the effect of pharmacological inhibitors.

T05-17B

A methodology for isolation and culture of adult astrocytes for Alzheimer's Disease research

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Astrocytes have active functions of paramount importance in the development and maintenance of the neural environment. Indeed, dysfunction of astrocytes has been linked to several neurodegenerative diseases, such as Alzheimer's Disease (AD), but the precise mechanisms are still unknown (Iram and Frenkel, 2012). These findings have led to a growing field of research of adult astrocytes during normal aging and neurodegenerative diseases in mouse models. While neonatal astrocytes are routinely isolated and cultured, techniques for effective isolation of astrocytes from the adult brainare very limited. One possible method requires transgenic expression of a fluorophore driven by an astrocytic promoter such as GFAP or ALDH1L1 and cell isolation using FACS sorting. However, the use of transgenic mice with labeled astrocytes becomes technically complex when investigating a disease mouse model as those mice most commonly have a lower breeding yield and the disease might lead to differential expression of the astrocytic promoter. Therefore, we developed a protocol that enables the culture of adult astrocytes from an AD mouse model. Adult brains are dissociated to a single cell suspension by papain-based enzymatic digestion. Next, the myelin is removed using a

percoll gradient. After several wash steps, red blood cells are lysed and the remaining cells are washed again and plated on PDL-coated wells. Cells are grown with complete Astrocyte Medium (ScienCell) and are let to proliferate for 1-2 weeks. On the 2^{nd}-3^{rd} weeks cell purity is assessed and subsequent experiments are conducted. The cells proliferate for 3 weeks and reach 20-30 times the initial cell number, thus enabling us to conduct more complex experiments with increased power and robust results. Astrocyte purity was measured over the 2^{nd}-3^{rd} weeks in culture by GFAP and Aldh1l1 immunostaining and by flow cytometry, with over 95% purity. We used this method to conduct various AD-related functional assays in astrocytes purified from adult WT and AD mice at several stages of the disease. Whereas this is not a direct, prospective method to purify astrocytes, this method enables investigation of highly enriched adult astrocyte cultures.

T05-20B

Early activation of microglia plays a central role in the disease pathogenesis of progressive myoclonus epilepsy, EPM1

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Progressive myoclonus epilepsy of Unverricht-Lundborg type (EPM1, OMIM 254800) is an autosomal-recessively inherited neurodegenerative disorder and it develops at an age of 6 to 16 years. Its presenting symptoms include tonic-clonic epileptic seizures and progressive, stimulus-sensitive and action-activated myoclonic jerks, which severely impair patients' everyday life. Later patients develop also other neurological symptoms, such as ataxia and dysarthria. EPM1 is caused by mutations in the cystatin B (*CSTB*, OMIM 601145) gene, which encodes the cysteine protease inhibitor CSTB. However, the physiological function of CSTB and the mechanisms underlying EPM1 are still not fully known. As a model for EPM1, mice deficient for CSTB (*Cstb-/-* mice) are commonly used as they recapitulate key clinical features of the disease, such as myoclonia and ataxia.

We have shown earlier that in *Cstb-/-* mouse cerebellum neurons are more sensitive to oxidative stress, leading to cell death mediated by cathepsin B. Furthermore, young *Cstb-/-* mice develop, in addition to neuronal hyperexcitability and neuron loss, also a non-neuronal brain phenotype. Already before the onset of clinical features, microglia cells in young *Cstb-/-* mouse brain are activated; however, their activation is dysregulated, which manifests as imbalance in the amount of pro-inflammatory and anti-inflammatory *Cstb-/-* microglia and as increased chemotactic activity, but reduced MHCII surface expression. In addition, *Cstb* mRNA expression is higher in microglia than in neurons or astrocytes and inflammatory response is induced in *Cstb-/-* mice cerebellum shown by expression profiling.

In this study, we aim to unravel the molecular basis underlying this early and dysregulated microglial activation in *Cstb-/-* mice by transcriptional profiling of *Cstb-/-* and control microglia from postnatal mice. Using a microarray-based approach, we identified 156 differentially expressed genes, which are enriched in immune response as well as in genes of the JAK-STAT signaling pathway. Moreover, we identified differential expression of 11 new genes previously not associated with EPM1 disease by RNA sequencing of *Cstb-/-* and control microglia. These genes suggest increased microglial activation, chemokine release, and dysregulated calcium-signaling in *Cstb-/-* microglia.

The identification of genes deregulated in *Cstb-/-* microglia performed in this study will provide insights into the consequences of *Cstb* deficiency on microglia and help to develop a more targeted treatment for EPM1.

T05-21B

Influence of autoimmune inflammation on remyelination in cuprizone-induced demyelination

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Background: Remyelination frequently fails in chronic multiple sclerosis (MS) lesions, yet it is usually successful in early lesions and experimental models. An anti-myelin immune response may contribute to remyelination failure in MS. In this respect, overexpression of IFNγ was shown to diminish remyelination in the cuprizone model. On the other hand, adaptive and innate inflammation was shown to increase remyelination in long-standing experimental demyelinated lesions.

Question/Methods: In order to determine the in vivo effects of a myelin-specific T cell response on remyelination, we combined cuprizone-induced de- and remyelination with active immunization with myelin oligodendrocyte glycoprotein (MOG) 35-55 and assessed the effect on oligodendroglial lineage cells and remyelination using light and electron microscopy.

Results: Immunization led to a marked T cell infiltration of the corpus callosum, an increase in IFNγ production and blood brain barrier breakdown. However, the density of oligodendroglia lineage cells was similar in immunized and non-immunized mice with cuprizone induced demyelination. Also, in situ hybridization for PLP revealed similar densities of myelinating oligodendrocytes. Importantly, no differences in the proportion of remyelinated axons were observed using histochemistry and electron microscopy.

Conclusion: We conclude that the remyelination response after cuprizone-induced demyelination is robust and not modulated by an additional adaptive immune response.

T05-22B

Inhibition of microglial activity is a major mechanism in neuroprotection of dopaminergic neurons by inhibition of Rho-kinase

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Introduction: Several recent studies have shown that activation of the RhoA/Rho-associated kinase (ROCK) pathway is involved in the MPTP-induced dopaminergic cell degeneration and possibly in Parkinson's disease. ROCK inhibitors have been suggested as candidate neuroprotective drugs for Parkinson's disease. The neuroprotective effects have been attributed to inhibition of the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced microglial response or to a direct protective effect on dopaminergic striatal axons. However, the mechanism responsible for the increased survival of dopaminergic neurons after treatment with ROCK inhibitors is not clear. This question must be clarified because ROCK inhibitors have been suggested as candidate neuroprotective drugs for Parkinson´s Disease and other brain diseases.

Materials and Methods: We exposed primary (neuron-glia) mesencephalic cultures (PMC), cultures of the MES 23.5 dopaminergic neuron cell line (MES 23.5) and PMC lacking microglial cells to the dopaminergic neurotoxin 1-methyl-4-phenylpyridinium (MPP^+) and different concentrations of the ROCK inhibitor, Y-27632, in order to study the effects of ROCK inhibition on dopaminergic cell loss and the length of neurites of surviving dopaminergic neurons. To obtain PMC lacking microglial cells, L-leucine methyl ester (LME) was used.

Results: In primary (neuron-glia) cultures, simultaneous treatment with MPP^+ and the ROCK inhibitor significantly reduced the loss of dopaminergic neurons. In the absence of microglia the ROCK inhibitor did not induce a significant reduction in the dopaminergic cell loss. The ROCK inhibitor induced a significant decrease in axonal retraction in primary cultures with and without microglia and in cultures of the MES 23.5 neuron cell line.

Conclusions: The results suggest: a) that ROCK inhibitors may provide a new neuroprotective strategy against PD and other brain processes with a major neuroinflammatory component; b) Inhibition of microglial ROCK is essential for the neuroprotective effects of ROCK inhibitors against cell death induced by the dopaminergic neurotoxin MPP^+; c) ROCK inhibition induced a direct effect against axonal retraction in surviving neurons.

T05-23B

Alterations of astrocytes proteome induced by beta-amyloid peptide: implications for Alzheimer disease pathogenesis

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In Alzheimer's disease (AD), astrocytes associate with deposits of β-amyloid (Aβ) peptide aggregates constituting the senile plaque and are known to take up Aβ. This interaction leads to the direct involvement of astrocytes in the neuronotoxic phenomenon as several alterations of the metabolic homeostasis and neuroprotective roles of astrocytes have been reported. To better understand the implication of astrocytes in the pathogenesis of AD, we have analyzed their proteome after treatment with the Aβ1-42 peptide surrogate Aβ25-35. The inactive inverse peptide Aβ35-25 was used as control treatment. Cultured astrocytes have been generated from P0 mouse cortex and have been treated with either 25 µM Aβ25-35 peptide for 48h or with 10 µM Aβ25-35 peptide for 8 days. They responded by a strong morphological response indicative of a reactive phenotype. Their protein profile has been analyzed by a comparative proteomic approach based on the 2D-DIGE methodology. Differentially expressed proteins in control and Aβ-treated astrocytes have been identified by mass spectrometry and the changes in expression of several of them have been validated by Western Blot. Apolipoprotein E is the protein whose expression is induced the most after treatment with Aβ25-35, followed by F-actin-capping protein subunit beta and three enzymes associated with the glycolytic pathway. Conversely, the Aβ25-35 peptide treatment causes a decrease in the expression of Peroxiredoxins and of actin isoforms, as well as of other actin-associated proteins, and of several mitochondrial proteins. Altogether, this work suggests that the acute activation of astrocytes by Aβ peptide induces an increased metabolic response and an augmented production of Apolipoprotein E, possibly as an attempt to neutralize Aβ peptide, contrasting with a decline in mitochondrial activity and OXPHOS, in anti-oxidant defenses and diminished actin cytoskeleton dynamics. Pending further confirmation, those observations may indicate a conflicting astrocytic response that could have adverse effects on neuronal survival and degeneration.

T05-24B

LIF haplodeficiency desynchronizes glial reactivity prolonging damage and functional deficits after a concussive brain injury

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When the nervous system sustains injury, the daily activities of glial cells are reprogramed by the actions of extracellular signals that are released in response to damage. Their responses are sophisticated and matched to the nature and severity of histopathology. Leukemia inhibitory factor (LIF) is rapidly induced in response to a spectrum of CNS and PNS injuries whereupon it stimulates receptors present on neurons, macroglia and microglia. Whether LIF signaling is, on balance, beneficial or detrimental for functional recovery has not been well established. Here we compared the extent of neocortical and subcortical white matter damage sustained by LIF haplodeficient mice vs. wild type mice using a closed head injury model in adolescent mice. Our studies reveal that both astrogliosis and microgliosis were comparatively diminished in the LIF haplodeficient mice acutely after injury (2 days), but exacerbated at later time points of recovery (7 and 14 days). This desynchronization of the gliotic response was accompanied by increased white and gray matter apoptosis, neuronal cell death (FluoroJade C), axonal degeneration (SMI-32) and hypomyelination (Rip). LIF haplodeficient mice also sustained greater callosal axonal loss and displayed more severe motor and sensory deficits at both 7 and 14 days of recovery compared to wild type mice. Altogether, these data demonstrate that LIF is an essential timing signal for both astrogliosis and microgliosis after brain injury, and that a 50% reduction in LIF expression is sufficient to cause a second wave of neurodegeneration and more severe neurological deficits. Supported by grant #CBIR13IRG017 from the NJ Commission on Brain Injury Research awarded to SWL.

T05-25B

Neuroprotective effect of pre-treatment with vitamin D against homcysteine-induced cellular dysfunction in cerebral cortex slices of rats

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Elevated plasma homocysteine (Hcy) has been detected in patients with various neurodegenerative conditions. Studies on neurons and cerebral tissue have revealed that hyperhomocysteinemia may inhibit mitochondrial electron transport chain (ETC) enzyme activity resulting in neuronal morbidity. As astrocytes convey a protective and supportive role towards neurons, is postulated that Hcy-induced astrocytic ETC inhibition might contribute to neurological dysfunction. Amounting new evidence has indicated that vitamin D plays a crucial role in brain development, brain function regulation and neuroprotection. The aim of this study is investigate the neuroprotective effect of pre-treatment of vitamin D in cerebral cortex slices incubated with mild concentration of Hcy. Cerebral cortex slices from adult rat are first pre-incubated per 30min with three different concentrations of vitamin D (50nM, 100nM and 250nM) and them we put Hcy per 1h to induce ETC dysfunction. After the incubation, the samples were washed, homogenized and storage in freezer - 80 °C to posterior analysis. Data showed that Hcy caused changes in parameters of bioenergetics (respiratory chain enzymes) and of mitochondrial function, affecting the mitochondrial mass, swelling the accompanied cell reduction of signs due to the low membrane potential. We analyzed neural cells by flow cytometry double labeled with Propidium Iodide (PI) and Hcy produce an increase in GFAP/PI cells but not affect NeuN/Pi cells. Homocysteine also induced oxidative stress, increasing the lipid peroxidation, reactive oxygen species generation and protein damage. An imbalance in antioxidant enzymes was also observed, where the system was not effective enough and thus producing hydrogen peroxide. Vitamin D reversed these alteration caused by Hcy. Our results show that homocysteine can lead to impair mitochondria function, induce astrogliose and induces changes in oxidative status in cerebral cortex, which were reverted by vitamin D. These findings suggest that the vitamin D may be a therapeutic strategy for complications caused by Hcy. However, more studies are necessary to understand the mechanism that Hcy-induced these alterations in astrocytes and how vitamin D prevents these effects.

T05-26B

Caffeine attenuates neuroinflammatory response and retinal ganglion cell loss in an ocular hypertension animal model

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Neuroinflammation and glial reactivity have been increasingly associated with the onset of glaucoma, the second leading cause of blindness worldwide. This degenerative disease is characterized by retinal ganglion cell (RGG) loss and optic nerve damage. Glaucoma is a multifactorial disease, but elevated intraocular pressure (IOP) is a major risk factor and the current treatments are mainly focused in reducing IOP. Still, many patients continue to lose vision despite the control of IOP and neuroprotective strategies aimed to prevent RGC loss are necessary.

Caffeine, an antagonist of adenosine receptors, is the most widely consumed psychoactive drug in the world and its consumption has been associated with reduced risk to develop neurodegenerative diseases. In fact, several evidences suggest that caffeine attenuates the neuroinflammatory responses and affords protection upon CNS injury.

In this work, we aimed to evaluate the potential neuroprotective effects of caffeine administration in an animal model with ocular hypertension (OHT).

OHT was induced in Sprague-Dawley rats by laser photocoagulation of limbar tissues. Caffeine (1 g/l) was administered in the drinking water, starting 15 days before OHT-induction. Animals were sacrificed 3 or 7 days after the induction of the OHT.

Caffeine decreased IOP of OHT animals, without altering IOP of control animals. Caffeine did not alter retrograde axonal damage in the optic nerve of OHT animals, as assessed by fluorogold tracing. Nevertheless, analysis of Brn3a-immunoreactive cells (RGCs) in retinal whole-mounts showed that caffeine significantly increased RGC survival after 7 days in OHT animals, compared to control animals. In addition, caffeine prevented OHT-induced alterations of inflammatory markers (IL-1β and TNF) and markers of microglial reactivity (CD11b and CD200) and phagocytic activity (TREM2), as assessed by qPCR.

These results show that caffeine administration controls the neuroinflammation and prevent RGC loss in OHT conditions. This study suggests that caffeine consumption or adenosine receptors antagonists might be a therapeutic option to manage RGC loss in glaucoma.

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T05-27B

Increased vulnerability to excitotoxicity in spermine oxidase overexpressing mouse: astrocyte-dependency

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Polyamine homeostasis plays a central role in a variety of intracellular processes. Recently, a transgenic mouse model conditionally overexpressing spermine oxidase in the neocortex neurons (Dach-SMO mouse) provided novel evidence of the complex network of functions carried out by polyamines in mammalian brain. To investigate on the mechanisms by which overexpressing spermine oxidase leads to increased vulnerability to excitotoxic brain injury and kainate-induced epileptic seizures, we performed immunocytochemical, neurochemical and electrophysiological evaluation together with assessment of the oxidative stress status in the cerebral cortex of spermine oxidase-overexpressing and control mice. In Dach-SMO mice we observed an increased number of astrocytes showing morphological features typical of reactive astrogliosis and a loss of neurons; a marked astrogliosis was confirmed by relative abundance of astrocyte processes and nerve terminals. Increased activity/expression of the antioxidant enzymes catalase and superoxide dismutase and of metallothionein-1, -2 and -3 in the cortex of Dach-SMO mice indicated a cellular oxidative stress involving both neurons and astrocytes. Induction of seizures by kainate in combined hippocampus-neocortex slices from Dach-SMO mice was inhibited by the astrocyte metabolism inhibitor fluoroacetic acid, indicating a role for astrocytes in the lowered threshold of the Dach-SMO mice to kainate neocortical seizures. Moreover, kainate evoked an unexpected release of the gliotransmitter glutamate in purified astrocyte processes prepared from Dach-SMO mice; use of AMPA and kainate receptor

agonists/antagonists indicated that the response to kainate was accounted for by AMPA receptors including Ca^{2+}-permeable GluA2-lacking receptors.

We conclude that reactive astrocytosis and activation of glutamate release from astrocyte processes might contribute, together with increased reactive oxygen species production, to the vulnerability to kainate excitotoxicity in mice overexpressing spermine oxidase. This mouse model with a deregulated polyamine metabolism would help to shed light on common pathways involved in excitotoxicity and on roles for astrocytes in increasing vulnerability to excitotoxic neuron injury.

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T05-28B

Regulation of the fractalkine ligand in human astrocytes

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The fractalkine ligand (CX3CL1) is expressed in astrocytes and reported to be neuro-protective. When cleaved from the membrane, soluble fractalkine (sCX3CL1) activates the receptor CX3CR1, which is expressed in neurons and microglia. The membrane bound form of CX3CR1 additionally acts as an adhesion molecule for microglia and infiltrating white blood cells. Here, the mechanisms involved in the up-regulation and cleavage of CX3CL1 from human astrocytes was investigated. A combination of ADAM17 (TACE) and ADAM10 protease inhibitors were found to attenuate TNFα, IL1β and IFNγ induced sCX3CL1 release in astrocytes. A specific ADAM17 inhibitor was unable to attenuate these effects, suggesting ADAM10 proteases induce release of sCX3CL1 from stimulated human astrocytes. A p38 MAPK inhibitor also attenuated the levels of sCX3CL1 upon treatment with either TNFα or IL1β. In addition, IKKα and IKKβ inhibitors significantly reduced the levels of sCX3CL1 induced by TNFα or IL1β in a concentration dependent manner, suggesting a role for the NFkB pathway. These findings are important for understanding the role of fractalkine in healthy and stimulated astrocytes and may benefit our understanding of this pathway in neuroinflammatory and neurodegenerative diseases.

T05-29B

Platelet derived growth factor and retinal neuroprotection: the impact on microglia

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Purpose: Microglia play a significant role in a number of neurodegenerative diseases, including glaucoma. Although over activation of microglia is often regarded as detrimental to neuronal health, evidence also suggests microglia may play a neuroprotective role.

We have recently shown that platelet-derived growth factor (PDGF), a neurotrophin present in the secretome of mesenchymal stem cells, protects against axonal damage in a rat model of glaucoma. Interestingly, in addition to a direct mechanism of neuronal protection, PDGF is also a potent chemoattractant for inflammatory cells.

We therefore investigated whether intravitreal PDGF injections could modify the inflammatory cell population in the glaucomatous eye. Due to the close relationship between neuronal synapses and glial activity we wished to explore whether microglia could protect against dendritic and synaptic changes that develop prior to retinal ganglion cell (RGC) loss.

Methods: Ocular hypertension was induced in SD rats using laser photocoagulation of the trabecular meshwork with subsequent intravitreal injections of 1.5 μg PDGF-AB or saline. Eyecups and retinas were processed for immunohistochemistry or qPCR after 2 weeks.

Wholemount immunolabelling was used to co-localise the phosphorylated PDGF-receptor with retinal cell types. Sagittal sections were used to identify microglia/macrophage number, morphology and location within the retina and optic nerve head (ONH). Confocal imaging enabled the quantification of synapses within the inner plexiform layer (IPL) whilst qPCR was used to investigate synaptic plasticity in addition to markers of monocyte migration and inflammatory cytokines.

Results: Intravitreal injection of PDGF increased the proliferation of the resident retinal microglia population and increased the recruitment of infiltrating monocytes. Interestingly, PDGF appeared to increase the proportion of microglia exhibiting a ramified phenotype and also increased retinal expression levels of IL-10 compared to saline injected eyes.

In parallel with these changes, PDGF treated eyes showed altered gene expression of the synaptic activity marker ARC and reduced glaucoma-related loss of synaptic punctae within the IPL.

Conclusion: Our results indicate that intravitreal injection of PDGF in a rat glaucoma model causes an elevated inflammatory response whilst also conferring robust neuroprotection to the RGC dendritic arbor. We propose that the altered monocyte population induced by PDGF represents a phenotype that may help limit synaptic loss.

T05-30B

Characterization of astroglial contribution to *C9ORF72* Amyotrophic Lateral Sclerosis (ALS) using patient-derived iPS astrocytes

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Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease characterized by rapid, progressive loss of motor function. Studies investigating the multigenic etiology of ALS have highlighted the death of upper and lower motor neurons as well as associated interneurons. However, mounting evidence indicates glial cells also play important roles in ALS by modulating disease onset and progression. In particular, astrocytes become reactive and exhibit altered morphology as well as functionality that can impede neuroregeneration.

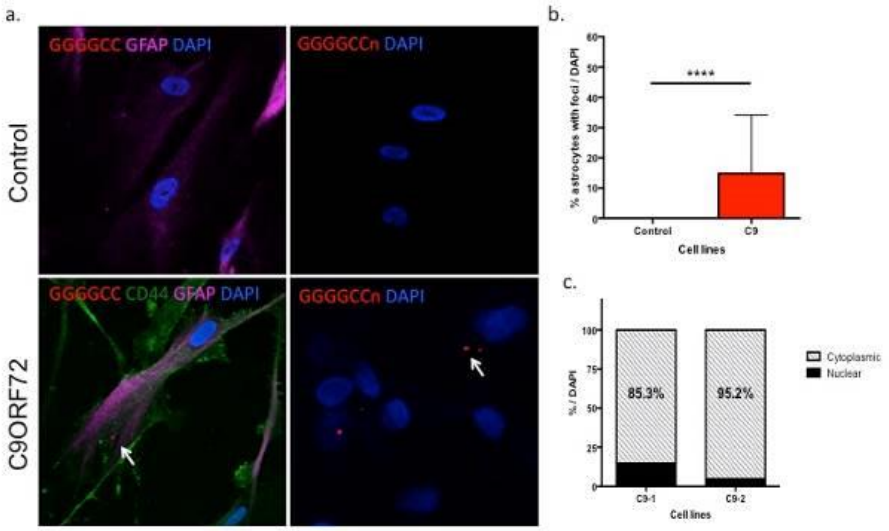
Recently, a (GGGGCC)n hexanucleotide repeat expansion in the noncoding region of the *C9ORF72* gene was identified as the most common genetic cause of ALS in both familial and apparent sporadic patients. Three potential mechanisms for motor neuron degeneration in *C9ORF72* ALS are haploinsufficiency, toxic RNA molecules containing the repeat expansion, and toxicity due to repeat-associated non-ATG (RAN) translation products. In this study, we sought to determine the extent of *C9ORF72* ALS pathology in patient-derived astrocytes and how they may contribute to the disease.

Patient fibroblasts were reprogrammed into induced pluripotent stem (iPS) cells, which were then differentiated into astrocytes (iPSA). Co-culturing iPSA with primary mouse cortical neurons upregulated expression of excitatory amino acid transporter 2 (EAAT2), an astrocyte-specific glutamate transporter tightly regulated by neurons in vivo. Functional sodium-dependent glutamate transport was also increased, but *C9ORF72* ALS iPSA demonstrated a blunted glutamate uptake response.

We next explored pathological consequences of the repeat expansion in the iPSA. *C9ORF72* iPSA expressed up to 90% less total *C9ORF72* mRNA than control iPSA as determined by quantitative real-time PCR. RNA fluorescent in situ hybridization showed accumulation of (GGGGCC)n RNA foci in roughly 15% of *C9ORF72* iPSA while control iPSA contained no foci. Unexpectedly, most of the (GGGGCC)n foci in *C9ORF72* iPSA were located in the cytoplasm rather than the nucleus, in contrast to our prior work on neurons. Poly-glutamine-proline RAN translation proteins were also detectable by ELISA in supernatants from *C9ORF72* iPSA cultures but not from control cultures. These data suggest that astrocytes may be affected in *C9ORF72* ALS and that iPSA can be used to determine their role in ALS pathogenesis. Ongoing studies are exploring potential mechanisms behind impaired astroglial

function and how they may converge on *C9ORF72* ALS pathology to result in toxicity to motor neurons.

Image



(GGGGCC)n RNA foci are detected in C9ORF72 iPS astrocytes, the majority of which are found in the cytoplasm. C9ORF72 iPS astrocytes have more foci than unaffected controls, which rarely have foci (a). On average, 14.8% of C9ORF72 astrocytes contained RNA foci (b). Of the C9ORF72 astrocytes that did have foci, the majority of foci were located in the cytoplasm (c). Two lines each of C9ORF72 and unaffected control lines were probed with RNA fluorescent in situ hybridization using (GGGGCC)x2.5 locked nucleic acid probes.

T05-31B

The neuroprotective role of microglia against amyloid beta toxicity in organotypic hippocampal slice cultures

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The development of Alzheimer's disease (AD) is highly associated with the presence of an inflammatory component. However, microglial cells, the innate immune cells of the brain, seem to play a complex role in the progression of AD with both, neurotoxic and neuroprotective properties. Organotypic hippocampal slice cultures (OHSC) serve as an excellent model system to investigate the interplay of glial and neuronal cells in the context of AD related neurodegeneration, since the *in vivo* formation of all cell types present in the hippocampal region is preserved. Aim of the present study was to investigate the effects of microglia depletion on amyloid beta-induced neurotoxicity in OHSC. Therefore, we performed a pharmacological treatment with clodronate (100 µg/ml) to entirely remove microglia from OHSC obtained from C57BL/6 mice (P1-4) and compared the effects of amyloid beta treatment with the effects in microglia-containing OHSC. Repeated treatment with amyloid beta for 7 days did not result in neuronal cell death in the presence of microglia, as detected by propidium iodide and NeuN co-staining. Interestingly, the ablation of microglia facilitated significant neuronal cell death in amyloid beta treated OHSC. Further, caspase cleavage after amyloid beta treatment was enhanced in OHSC depleted from microglial cells compared to control slices containing resident microglia. Additionally, we investigated the microglial properties after amyloid beta treatment in OHSC. Microglia kept their ramified morphology but secreted cytokines (IL6, TNFalpha) as measured by ELISA and, further, showed elevated nitric oxide production, as detected using the Griess assay.

In summary, our study argues for a beneficial role of functional microglia acting against formation of neurotoxic forms of amyloid beta and supporting neuronal resilience in this in situ model of AD pathology.

T05-32B

Lysyl oxidase is a novel target of lithium that regulates astrogliogenesis in adult CNS white matter

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Lithium is the main treatment for Bipolar Disorder (BD) and is a potential therapy for multiple neurodegenerative diseases, including Alzheimer's disease (AD). Notably, white matter (WM) abnormalities are a key feature of BD and AD, although the underlying pathophysiological causes are unresolved. Astrocytes have multiple homeostatic functions in the CNS and are essential for WM integrity. Here, we examined whether astrocytes are targets of lithium treatment in CNS white matter. Adult GFAP-EGFP mice were killed humanely in accordance with the Home Office Animals (Scientific) Act 1986 (UK), and optic nerves isolated with retina intact. Nerves were maintained *ex vivo* in organotypic culture in control medium or medium containing 20 mM lithium chloride. After 3 days *in vitro*, optic nerves were analysed by confocal microscopy, immunohistochemistry, microarray, and qRT-PCR. Lithium treatment had striking effects on astrocytes, doubling their number and inducing the development of a unique highly polarised astrocyte phenotype. Microarray analysis identified lysyl oxidase (Lox) as a major target of lithium and qRT-PCR confirmed that lithium treatment almost completely abolished expression of Lox in the optic nerve. Furthermore, incubation of optic nerves with the recognised Lox inhibitor BAPN (β-aminopropionitrile, 4mM) had striking morphogenic effects on astrocytes equivalent to those observed in lithium. The results identify the enzyme Lox as a novel target of lithium and profound regulator of astrocyte morphology, which may have implications for neurodegenerative diseases.

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T05-33B

Specific expression of the neurotoxic microRNA family *let-7* in the cerebrospinal fluid of patients with Alzheimer's disease

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MicroRNAs (miRNAs) are small non-coding single-stranded RNAs that are involved in post-transcriptional regulation of eucaryotic gene expression. However, we demonstrated recently that extracellularly delivered individual members of the *let-7* miRNA family, which are abundantly expressed in the adult mammalian brain, can also serve as endogenous signaling molecules stimulating both microglia and neurons and leading to neuroinflammation and neurodegeneration through the Toll-like receptor signaling pathway. We report here that human cerebrospinal fluid (CSF) contains a specific pattern of *let-7* miRNAs, as determined by quantitative real-time PCR. Whereas let-7a, let-7b, and let-7e were detected abundantly at similar levels in CSF of patients with Alzheimer´s disease (AD) and control individuals, let-7c, let-7g, and let-7i copy levels were only low. Let-7d, let-7f, and miR-98 were hardly or not present, respectively. Further, let-7b and let-7e were specifically released during the course of AD since CSF from patients with AD contained significantly increased copy numbers of these miRNAs compared to control individuals. However, this increase in copy

numbers was not seen in other neurodegenerative diseases such as fronto-temporal dementia. In the CSF of AD patients, both miRNAs were found to be associated with exosomes, as assessed by quantitative real-time PCR and western blot. All tested members of the *let-7* family induced neurotoxic effects in cultures of primary neurons derived from C57BL/6 mice, although to a varying extent. These neurodegenerative effects induced by *let-7* miRNAs did not require the presence of non-neuronal cells as shown by examination of the neuroblastoma cell line N1E-115.

In summary, our results suggest that the miRNAs let-7b and let-7e present extracellularly in human CSF may offer utility as biomarkers of AD.

T05-34B

Caffeine modulates retinal neuroinflammation and cell survival in retinal ischemia

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Retinal ischemia is a common cause of visual impairment and blindness and plays an important role in the pathophysiology of several retinal disorders, namely ischemic optic neuropathy, diabetic retinopathy and glaucoma. The retinal cell death and inflammation have been well described after retinal ischemic episodes, and neuroprotection may be a valuable therapeutic strategy to manage disease. Mounting evidence demonstrates that caffeine attenuates inflammatory responses and affords protection upon CNS injury.

The aim of this work was to investigate whether caffeine intake prevents retinal neuroinflammation and cell death induced by retinal ischemia-reperfusion (I-R) injury in rats.

Caffeine was administrated in the drinking water (1 g/l) two weeks before ischemia induction and throughout the experiment. Retinal ischemia was induced in one eye by increasing intraocular pressure (IOP) for 60 min. The animals were sacrificed after 24 h or 7 days of reperfusion. The contralateral eye served as the control eye.

Caffeine intake was 125±7 mg/kg/day and serum caffeine concentration was 55 μM (2 weeks consumption) and 98 μM (3 weeks consumption). Caffeine administration did not alter IOP throughout the study.

At 24 h post-ischemia, caffeine exacerbated microglia reactivity, without significant changes in the levels of TNF and IL-1β. In addition, caffeine significantly enhanced the number of TUNEL-positive cells induced by I-R injury. However, at 7 days post-ischemia, the number of reactive microglia, the levels of TNF and IL-1β and the production of reactive oxygen species were inhibited in the I-R retinas of caffeine-drinking animals. Moreover, the number of retinal TUNEL-positive cells was significantly reduced in caffeine-treated animals, compared to animals drinking water.

These results suggest that caffeine modulates neuroinflammation and at long term it may afford neuroprotection to the retina against damage induced by I-R injury.

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T05-35B

Mechanism of nimodipine-dependent inhibition of amyloid b stimulated interleukin 1-beta production from microglia

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Background: The activation of microglia by amyloid b (Aβ) and the increased production of interleukin 1-beta (IL-1β) are considered two early phenomenon in the pathogenesis of Alzheimer's disease. We have recently demonstrated in vitro and in vivo that Aβ triggers IL-1β release by activating the P2X7 receptor (Sanz et al., J Immunol, 2009, 182, 4378) and that nimodipine is able to inhibit this process (Sanz et al., Br J Pharmacol. 2012; 167(8):1702-11.). Nimodipine, a dihydropyridine L-type calcium channel blocker, has been mainly used in treatment of cardiovascular diseases, recently it has been found to be beneficial in many central nervous system diseases such as brain injury and vascular dementia, but the mechanism involved in neuroprotection is not clear. The scope of this study was to evaluate the mechanism of nimodipine-dependent inhibition of IL-1β production induced by Aβ.

Methods: Microglial cells, N13 or primary mouse microglial cell, were incubated for 4 days with Aβ in absence or presence of nimodipine. Supernatant was used to measure extracellular IL-1β, while the cellular lysate was used to measure intracellular IL-1β, NALP3 expression, caspase-1 activity and intracellular ATP caspase-1 activity.

Results: Nimodipine significantly decreased IL-1β production, NALP3 expression, caspase-1 activation and intracellular accumulation of ATP triggered by Aβ.

Conclusions: We show that nimodipine caused inhibition of inflammasome activation by decreasing NALP3 expression and caspase-1 activity. Nimodipine might contribute to decrease the inflammatory state characteristic of the patients with Alzheimer disease.

T05-36B

Demyelination induces functional deficit in the non-human primate optic nerve

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Multiple sclerosis (MS) is a chronic inflammatory demyelinating disease of the central nervous system (CNS). Loss of myelin is associated with an inflammatory response and results in a progressive loss of axons and the development of chronic and irreversible neurological deficits. In particular, lesions of the **optic system** occur in about 70% of patients, leading to altered vision and sometimes blindness. Since re-myelination provides neuro-protection and insures proper saltatory conduction, one major goal in MS, aside reduction of the CNS inflammatory infiltrates, is to favour myelin repair. This can be achieved either by favouring endogenous repair by activation of the endogenous pool or by promoting re-myelination through cell engraftment. **Animal models** of demyelination induced by toxin are suitable to study of mechanisms of demyelination-re-myelination and the development of approaches aiming at promoting CNS re-myelination. Lysolecithin (LPC) induced demyelination in non-human primate optic nerve leads to chronic demyelination (Lachapelle et al 2005). **However, the consequence of demyelination on visual function is unknown.** Visual evoked potentials (**VEP)** can provide important diagnostic information regarding the functional integrity of the visual system including MS patients. VEPs are used to measure the functional integrity of the visual pathways from retina via the optic nerves to the primary visual cortex of the brain in monocular and binocular conditions. The pupillary light reflex (**PLR**) is a reflex that controls the constriction and dilation of the pupil, in response to light intensity in different color stimulation and/or spontaneous or inducible conditions. Our data show that LPC-induced demyelination of the macaque optic nerve, altered **non visual and visual systems**. Demyelination induced a delay in VEP latency and a decrease of amplitude in binocular condition andVEP abnormalities in monocular condition in response to a black and white checkerboard. Further more, PLR revealed a drastic abolition of pupil constriction and redilation.This model of demyelination with functional deficit of the macaque optic

nerve, will help our understanding of the consequence of long-term demyelination on visual functions. Moreover, it appears as an essential step towards clinical trials in providing the necessary information on security, toxicity, tolerance and efficacy of cell- or pharmacology-based therapy to promote myelin repair. Supported by “Investissements d’Avenir” ANR-10-IAIHU-06.

T05-37B

Astrocytes increase fatty acid oxidation following traumatic brain injury in the developing brain

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Traumatic brain injury (TBI) is the leading cause of mortality and permanent life-long disability in children. It is characterized by deficits in cognition, attention, and sensorimotor integration. Evidence suggests that depressed cerebral energy metabolism is a major attribute of TBI. However, the mechanisms of impaired cerebral energy metabolism and secondary energy failure remain poorly understood.

Although glucose is the primary substrate for brain energy and metabolism, the brain also has the capacity to oxidize fatty acids. In this study, we first demonstrate *in vivo* that: 1) only astrocytes express carnitine palmitoyltransferases (mitochondrial CPT1A and CPT2) which enables them to utilize long-chain fatty acids for energy and metabolism; 2) there is regional heterogeneity in CPTs expression with the hippocampus being significantly higher than the cortex; and 3) the expression of CPTs changes during brain development, with highest expression between post-natal days (PND) 21-28.

Using controlled cortical impact as a clinically relevant model of focal moderate-to-severe brain trauma, we used PND 21 rats and determined that the expression of CPTs were increased in the ipsilateral hippocampus compared to contralateral and sham operated animals at 6hrs and 24hrs post TBI. [1-^{14}C]oleic acid oxidation was significantly increased (1.5 fold increase at 6hrs and 2.2 fold increase at 24hrs) in the ipsilateral hippocampus following TBI compared to the contralateral hippocampus and sham operated animals. No changes were observed in the cortexes following TBI. Further experiments are being conducted to determine whether increased fatty acid oxidation contributes to improved cellular and behavioral outcomes. The unique ability of astrocytes to utilize fatty acids for energy and metabolism make it a candidate to develop cell specific targeted therapies following brain injury.

T05-38B

Low molecular weight polysialic acid shows anti-inflammatory effects on human THP1 macrophages

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Phagocytes show an over-activated complement-phagosome-NADPH oxidase (NOX) signaling pathway in Alzheimer disease. Polysialic and oligosialic acids are glycans composed of sialic acid monomers, which are attached to the outermost ends of lipids and proteins on the surface of healthy brain cells. These structures are recognized by sialic acid-binding immunoglobulin-like lectin (Siglec) receptors of microglia and macrophages, which show immunoreceptor tyrosine-based inhibitory motif (ITIM)-signaling and counteract the complement-phagosome-NOX signaling pathway.

Here we show that low molecular weight polysialic acid with average degree of polymerization 20 (polySia avDP20) binds to recombinant SIGLEC11/Fc-fusion protein. *In vitro,* the human macrophage cell line THP1 was used as a model system of SIGLEC11 expressing cell, which is a putative receptor for polysialic acid (polySia). We show that polySia avDP20 acts anti-inflammatory on human THP1 macrophages by inhibiting the lipopolysaccharide induced gene transcription of tumor necrosis factor-

a (TNF-α), while knockdown of SIGLEC11 eliminate this effect. Furthermore, polySia avDP20 slightly reduced phagocytosis of amyloid-β fibrils and debris. In addition, polySia avDP20 completely prevented the associated reactive oxygen species production by stimulated macrophage cells, an effect that was as strong as known superoxide scavengers like Trolox and superoxide dismutase-1 (SOD1). By using an *in vitro* human neuron-macrophage co-culture system, amyloid-β treatment resulted in neurotoxicity with loss of neurites that was abrogated by treatment with polySia avDP20. Furthermore, in a systemic inflammatory *in vivo* model of humanized SIGLEC11 transgenic mice, polySia avDP20 inhibited lipopolysaccharide (LPS)-induced gene transcription of TNF-α.

In total, data show that polySia avDP20 binds to human SIGLEC11 and acts anti-inflammatory on SIGLEC11 expressing macrophages.

T05-39B

Effect of long-term paroxetine treatment on Ab pathology and microgliosis in the $APP_{swe}PS1_{\Delta E9}$ mouse model of Alzheimer's disease

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Question: Alzheimer's disease (AD) is frequently associated with depression, and like depression, AD is characterized by impaired serotonin neurotransmission. Recently, prophylactic treatment with a selective serotonin reuptake inhibitor (SSRI) was found to reduce Ab load in the $APP_{swe}/PS1_{\Delta E9}$ transgenic (Tg) mouse model of AD (Cirrito et al. 2011. PNAS 108:14968). Here, we asked if therapeutic SSRI treatment of Tg mice could reduce or impede the age-dependent increase in Ab pathology, possibly by modulating microglial capacity to take up and clear Ab.

Methods: Male Tg and littermate wildtype (Wt) mice were treated orally from 9-18 month of age with either paroxetine (Seroxat®, 5 mg/kg) or vehicle. To establish a baseline, groups of 3 and 9 months old Tg and Wt mice were also included. Paroxetine levels in serum were measured by mass spectrometry. Brains from PFA perfused mice were processed into 50 mm thick free-floating sections. For estimation of % Ab plaque load and number of microglial cells, sections were stained with 6E10 or Iba-1 antibody, respectively. Quantifications were performed using the NewCast™ software by either grid or optical disector counting.

Results: Estimation of % Ab plaque load showed a persistent and significant increase from 3-9 month (0.04±0.04% (mean±SD) vs. 8.1±0.7%; $p<0.05$), and from 9-18 month (8.1±0.7% vs. 16.3±4.3%; $p<0.001$) in neocortex of vehicle-treated Tg mice. Unexpectedly, Tg mice treated with paroxetine or vehicle for 9 months showed a comparable % Ab plaque load (14.8±2.9% vs. 16.3±4.3%; $p=0.18$; n=9-14). The number of Iba-1^+ microglia showed a 2-fold increase in Tg vs. Wt mice in both treatment groups ($p<0.01$, both comparisons), with no effect of paroxetine treatment on the number of Iba-1^+ microglia in the Tg mice ($p>0.05$). Paroxetine levels in serum were within therapeutic range.

Conclusions: These results, which are still preliminary, suggest that treatment with paroxetine has no effect on Ab deposition in the neocortex when the Ab pathology is already established at the time of treatment start. Furthermore, paroxetine treatment appears to have no effect on the Ab-induced microgliosis or microglial clearance of Ab.

T05-40B

Alzheimer's amyloid degradation by secreted lysosomal enzymes

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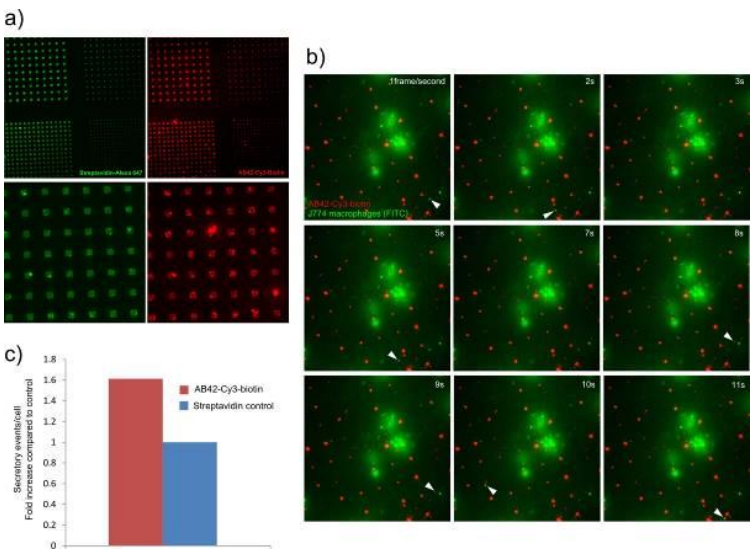
In Alzheimer's disease (AD), some brain regions associated with neurodegeneration present abundant amyloid-beta (Aβ) fibrillogenesis. It is unclear how microglia or macrophages in the brain can proteolytically degrade Aβ fibrils (fAβ) and plaques that are significantly larger than these cells. A new possible mechanism is suggested by an earlier study published by our lab in which it was found that macrophages could degrade very large aggregates of LDL by creating an *extracellular,* acidic compartment that we called the "lysosomal synapse" into which lysosomal contents are secreted (Haka et *al*. 2009).

To study the interaction of microglia and macrophages with fAβ, and in collaboration with the Cornell NanoScale Science & Technology Facility (CNF), we fabricate surfaces to present spatially defined fluorescently labeled streptavidin on glass cover slips which are thereafter incubated with Aβ-Cy3-biotin fibrils of 100-200nm in length. Biotinylated fAβ is captured by the multivalent streptavidin, thus immobilizing fAβ onto the glass surface (Fig. A). Macrophages and microglia, for which their lysosomes were previously labeled with FITC-dextran, are subsequently incubated on the fAβ-coated micro-pattern and imaged using Total Internal Reflection Fluorescence Microscopy (TIRFM).

TIRFM reveals rapid FITC flashes (indicated by white arrowheads in Fig. B) at the pattern surface which are indicative of lysosomal content release. As FITC exits the lysosomes, it undergoes fluorescence increase associated with the transition from the acidic lysosomal environment to the more alkaline pH of the extracellular environment. Measurements from a reduced number of samples indicate that macrophages incubated on fAβ-coated surfaces present higher secretory activity when compared to those exposed to streptavidin-coated surfaces (Fig. C).

The proposed studies are aimed at exploring new ground for treatments based on the use of therapeutic agents to increase lysosomal activity. These could potentially lead to an enhancement in microglia fAβ lysosomal degradation and clearance.

Image



T05-41B

Traumatic brain injury in the mouse leads to proliferation of oligodendrocyte progenitor cells in important white matter tracts

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White matter injury as well as diffuse axonal injury are important consequences of traumatic brain injury (TBI) and associated with a poor patient outcome. Injury to axons, myelin and death to the myelin-producing oligodendrocytes leads to dysfunction of neuronal signaling. Endogenous repair mechanisms are limited in the central nervous system and even though oligodendrocyte progenitor cells (OPCs) are present in the adult brain, little is known about the proliferative activity of these cells and the possible regeneration of functional myelinating oligodendrocytes post-TBI. The present study aimed to investigate proliferation of OPCs in a TBI model of diffuse axonal injury in the mouse from two to 21 days post-injury. Midline fluid percussion injury was used as injury model and proliferation was detected by 5-ethynyl-2'-deoxyuridine (EdU) labeling and quantified with immunohistochemical staining for OPC markers in several white matter loci. The corpus callosum, external and internal capsule and fimbriae were thoroughly investigated at three bregma levels in each animal and proliferation of OPCs was analyzed. Quantification of EdU/DAPI/Olig2-positive cells showed an increased number of triple positive cells in the TBI group when compared to sham-injured controls at 2 and 7 days post-injury ($p \leq 0.05$ in all evaluated regions at day 7) which had subsided at 21 days post-injury. Triple positive EdU/DAPI/NG2 staining confirmed an increased proliferation of OPCs at 7 days post-injury. The importance of OPCs proliferation following TBI is yet unclear. We hypothesize that it implies a regenerative attempt which may lead to enhanced replacement of lost oligodendrocytes and re-myelination of injured white matter tracts following TBI.

T05-42B

siRNA screen of microglia to identify neuroprotective drug targets in Parkinson´s disease

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Question: Neuroinflammation is a fundamental process contributing to the death of dopaminergic neurons in Parkinson´s Disease (PD). During this process, activated microglia secrete cytotoxic substances which lead to neuronal death. Therefore we are looking for the molecular mechanism that reverses the inflammatory activation of microglia, since this knowledge would be essential to protect from neurodegeneration.

Methods and Results: Very interestingly our previous data (Neubrand et al., 2014) indicate that adipose derived mesenchymal stem cells (ASCs) exert important anti-inflammatory actions on microglia. We observed that microglia exposed to ASCs or their secreted factors (conditioned medium, CM) underwent a dramatic cell shape change into a highly elongated morphology (Fig 1A), similar to the phenotype of microglia observed in a healthy brain. The elongation induced by ASCs was associated with a decrease of the pro-inflammatory cytokine TNFalpha (Fig 1B) as well as with an upregulation of neurotrophic factors. Thus, ASC stimulated microglia represent an ideal tool to study the intracellular events necessary for the transition from inflammatory activated to non-inflammatory neuroprotective microglia. In this way we have already identified the small RhoGTPases Rac1 and Cdc42, which are important regulators of the actin cytoskeleton, as essential molecules in this transition (Fig 1C).

Since these molecules represent possible drug targets to induce the reversion of neurotoxic microglia to neuroprotective ones, we are currently performing an siRNA screen to identify the molecular players of this ASC-induced reversion. Because this transition is easily detectable by light microscopy (see Figs 1A and C) and changes in the cell shape are intrinsically related to changes of the cytoskeleton, we are carrying out a microscopy-based screen of the major cytoskeletal regulators. In addition, we are including in the screen the regulators of microglia-specific activation/inflammatory pathways as siRNA targets.

Conclusion: Our project is the first siRNA screen performed in primary microglia and we aim to identify a list of molecules that are specifically implicated in the reversion from activated to neuroprotective microglia. Since positive hits would represent potential neuroprotective drug targets, the outcome of this screen opens up a variety of novel investigation lines and therapies in PD or other neurodegenerative diseases.

Image

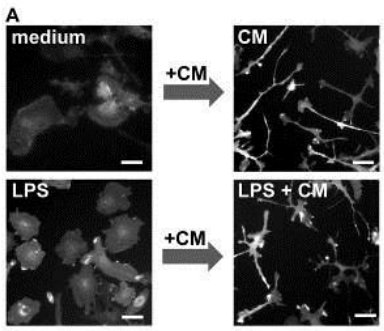


Fig 1A: Microglia underwent a dramatic cell shape change when treated with ASC CM, even in the presence of the inflammatory bacterial endotoxin lipopolysaccharide (LPS). Bars = 10mm

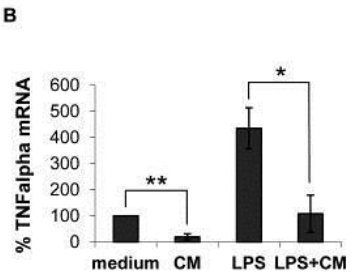
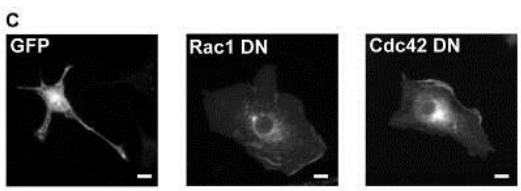


Fig 1B: Gene expression of the inflammatory cytokine TNFalpha was quantified by qRT-PCR. Mean+/-SEM. *p<0.05, **p<0.001



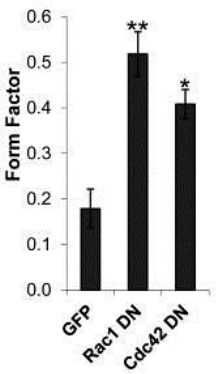


Fig 1C: GFP as negative control and the dominant negative (DN) mutants of the small RhoGTPases Rac1 and Cdc42 were transfected into microglia and treated with CM. Both mutants inhibited the ramification of microglia, as quantified with the form factor (right panel). Bars = 10mm; Mean+/-SEM. *p<0.05, **p<0.001

Poster topic 06
Extracellular matrix and cell adhesion molecules

T06-01A

Astrocytes as a crossroad for plasminogen activation

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Tissue-type plasminogen activator (tPA) catalyses the activation of the inactive plasminogen to the extracellular protease plasmin. This plasminogen activation system plays multiple roles in the CNS, such as regulation of the extracellular matrix, cell migration, degradation of fibrin deposits or neurotransmission. These processes are involved in physiological processes, such as learning and memory, as well as pathological conditions, such as neuroinflammation or cell death. Here, we show that astrocytes regulate the plasminogen activation system at different levels:

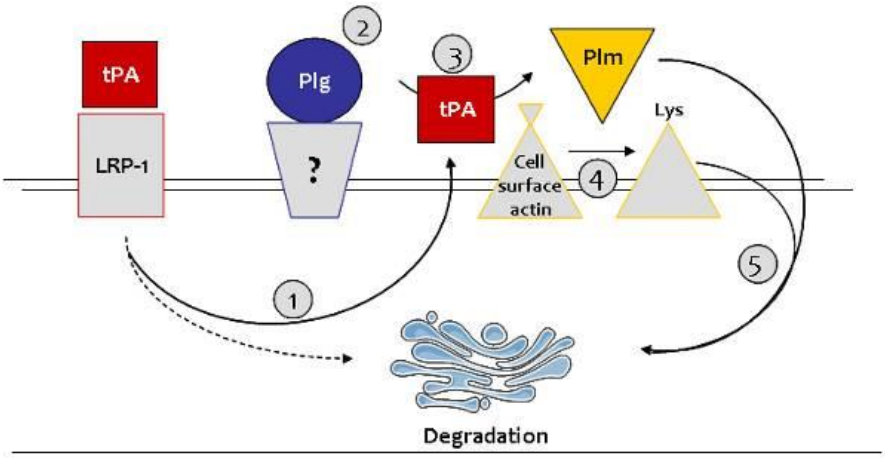
First, astrocytes can recycle tPA: tPA released by neurons is constitutively endocytosed by astrocytes via the low-density lipoprotein-related protein receptor, and is then exocytosed in a regulated manner. This mechanism of recycling of tPA by astrocytes is regulated by extracellular glutamate via Kainate receptor-induced signalling pathway involving protein kinase C. In turn, tPA regulates glutamatergic signalling by acting on NMDA receptors.

Second, astrocytes can serve as a surface for plasminogen activation: plasminogen activation by tPA is enhanced in the presence of astrocytes.

Third, astrocytes can uptake and degrade plasmin: plasmin is endocytosed by astrocytes and targeted to the lysosomal comportment. The endocytose of plasmin is triggered by its proteolytic action on cell surface actin: cell surface actin, acting as a substrate for plasmin, can drive plasmin clearance, thus avoiding excessive proteolytic activity at the cell surface.

Thus, astrocytes can exert a fine regulation of plasminogen activation by recycling tPA, promoting plasminogen activation and removing excessive plasmin for the cell surface. Given the multiple functions proposed for the plasminogen activation system in the CNS, this mechanism could interfere with various processes important for brain functions in health and diseases.

Image



tPA is taken up by astrocytes via LRP-1 mediated endocytosis, leading to degradation and recycling (1). Plasminogen (Plg) binds to an unknown receptor at the surface of astrocytes (2), facilitating conversion into plasmin (Plm) by tPA (3). Plm activity liberates a lysine on cell surface actin (4) which can interact with the lysine binding site of Plm. The binding of Plm to cell surface actin is necessary for its uptake by astrocytes, finally leading to its degradation (5).

T06-02A

The expression of a type-4 disintegrin and metalloproteinase with thrombospondin motifs (ADAMTS-4) in the oligodendrocyte lineage

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Chondroitin sulfate proteoglycans (CSPGs) are an important component of the extracellular matrix where they have roles in development and plasticity in the normal central nervous system (CNS). After a CNS injury, CSPGs are upregulated and become a major inhibitory component for axonal regeneration and myelination. ADAMTS-4 (a type 4 disintegrin and metalloproteinase with thrombospondin motifs) is a protease of the extracellular matrix that has the ability to degrade CSPGs such as neurocan, versican, aggrecan and versican. In our lab, we recently showed a beneficial effect of ADAMTS-4 on axonal regeneration and function recovery after a spinal cord injury in the rat (Lemarchant *et al.,* 2014). However, the localization and roles of ADAMTS-4 have only been poorly described in the normal CNS. To characterize the pattern of expression of ADAMTS-4, we used in the present study transgenic mice at early development stages and in the adult. In these mice, a bacterial lacZ gene was inserted as a reporter, so that the endogenous ADAMTS-4 gene promoter drives the expression of β-galactosidase. Immunohistochemistry (IHC) of β-galactosidase on brain and spinal cord sections of ADAMTS-4 knock-out/LacZ knock-in mice showed that ADAMTS-4 is strongly expressed in the central nervous system. Interestingly, ADAMTS-4 expression was not detected in neurons, astrocytes, endothelial cells or microglia but was restricted to oligodendrocytes. Moreover, ADAMTS-4 appears at late stages of the oligodendrocyte lineage differentiation as observed *in vivo* by IHC and *in vitro* in rat oligodendrocytes cultures. Our data provide new information on ADAMTS-4 expression in the CNS, opening new leads of research to further describe its effects. Likewise, the failure of neurorepair after a CNS injury, caused by CSPGs accumulation, could present a new field of experiments involving ADAMTS-4.

T06-01B

Investigation of oligodendrocyte differentiation in the inhibitory multiple sclerosis lesion microenvironment *in vitro*

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In multiple sclerosis (MS), oligodendrocyte precursor cells (OPCs) migrate to lesion sites to repair damaged myelin. Throughout MS disease progression, the ability to repair such damage diminishes considerably. This reduced capacity to regenerate is thought to be a consequence of lesion-associated inhibitory factors (LAIFs) that perturb OPC maturation into myelinating oligodendrocytes (OLs). These LAIFs include, but are not limited to, chondroitin sulfate proteoglycans (CSPGs) and myelin debris. The current study aims to further characterize the OL response to CSPG exposure, as well as explore the molecular pathways involved in CSPG-mediated inhibition of OL differentiation and myelination. We have validated the impact of CSPGs on OL maturation using a primary mouse OL culture system. Exposure to CSPGs impaired the ability of the OL to extend processes, and also negatively impacted the size of the myelin sheet produced in culture. In hopes of expanding the characterization of OL response to CSPG exposure, a CSPG spot assay technique is used. This method produces a microenvironment more highly reminiscent of CSPG deposition in the MS lesion than standard culture conditions. This technique is used to investigate the impact of CSPG exposure on OLs at varying stages of development. Additionally, this work explores the potential involvement of glycogen synthase kinase 3b (GSK-3b) and Akt signaling in mediating the inhibitory effects of CSPG exposure on OL morphological differentiation. Results of this study will provide a better understanding of how the lesion microenvironment contributes to MS pathophysiology, and elucidate avenues to promote remyelination within the inhibitory milieu.

Poster topic 07
Gene expression and transcription factors

T07-01A

Human microglia transcriptome and cross-species analysis

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Microglia are the primary immune cells of the central nervous system (CNS).They play an important role in the maintenance of tissue homeostasis, support and neuroprotection, constantly surveying their environment and quickly responding to homeostatic perturbations. In addition to their physiological role, microglia are increasingly implicated in neuropathological and neurodegenerative conditions, such as Alzheimer's disease, Parkinson's disease and glioma progression. The development of high-throughput RNA sequencing (RNA-seq) has allowed for highly sensitive, large-scale assessments of transcript sequences and expression levels across tissues and conditions. In rodents, transcriptome analysis of pure populations of microglia has contributed to our understanding of the uniqueness regarding these cells, how they differ from other myeloid cells, such as macrophages and dendritic cells, as well as contribute to elucidate the mechanisms underlying the changes occurring during development, aging and disease mechanisms. Here we report on the generation of microglia transcriptome across species. We have optimized microglia isolation protocols for human, rhesus macaque and mouse that yield a high purity microglia population (up to 98% purity). This acute isolation protocol is based on mechanical dissociation and a two-step density gradient purification, followed by CD11b/CD45 staining and FACS sorting analysis. RNA-sequencing was applied to microglia isolated from mouse, macaque and human cortex and a cross-species transcriptome analysis was set up to find common and unique genes and pathways. The generation of human microglia transcriptome and its comparison to macaque and mouse microglia will be presented, shedding light on microglia divergence across species, as well as possible unique functions that differ between primate and non-primate species.

T07-02A

Phosphorylation state of ZFP191 regulates maturation of late-stage oligodendrocytes

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Myelin is a multilayered membrane that wraps axons. It provides protection to the axons and allows for the rapid conduction of action potentials along the nerves. In the brain and spinal cord (central nervous system), myelin is formed by oligodendrocytes (OLGs). Previous work from our laboratory demonstrated that ZFP191 is critical for CNS myelination: *Zfp191* null mutants are severely hypomyelinated despite the presence of normal numbers of mature OLGs (Howng et al., 2010 Genes Dev 24(3):301-311). Our results demonstrate that the phosphorylation state of ZFP191 changes as OLGs mature from their progenitors. In oligodendrocyte progenitor cells (OPCs), the majority of the protein is phosphorylated, but as the cells differentiate to mature myelinating OLGs, the non-phosphorylated form of ZFP191 accumulates. We have also identified the DNA motif to which ZFP191 binds, and strikingly, we have detected binding sites in proximity to several genes that are crucial for maturation of OLGs. Our data suggest that the changes in ZFP191 phosphorylation state results in changes in ZFP191 DNA binding capability: non-phosphorylated ZFP191 has a greater capacity to

bind its target DNA sequence than does the phosphorylated form. Based on these finding we have used site direct mutagenesis to demonstrate that only a mutant that resembles the non-phosphorylated form of ZFP191 was able induce OLG maturation. We suggest that changes in the phosphorylation state of ZFP191 control the maturation of oligodendrocyte lineage cells and the potential of OLGs to myelinate axons. Importantly, this provides a new potential target for intervention to enhance myelination and remyelination.

T07-03A

Axonal and presynaptic RNAs are synthesized in the nearby glial cells

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Axons and nerve endings are known to contain cytoplasmic systems of protein synthesis that contribute to their maintenance and plasticity. To investigate the origin of the involved RNAs, experiments were made with squid giant axons and with presynaptic synaptosomes from the squid optic lobe. The experiments demonstrated that RNA species supporting the local translation processes are synthesized in their surrounding glial cells.

Indeed, when internally perfused giant axons were incubated with [^{3}H]uridine, radiolabeled RNA appeared in the axon perfusate within a few minutes. Notably, its content markedly increased upon addition of agonists of different receptors selectively present in the surrounding glial cells. Conversely, pretreatment of the perfused axon with antagonists of these receptors prevented these effects. Sedimentation analyses of the newly synthesized perfusate RNA indicated the large prevalence of several low MW species and of sizable amounts of RNAs associated with ribosomal subunits.

Likewise, when slices of squid optic lobe were incubated with [^{3}H]uridine, most newly synthesized RNA was present in the large synaptosomes derived from the nerve terminals of retinal photoreceptors. The retinal location of their cell bodies proved that its synthesis occurred in perisynaptic cells, presumably glial cells. Sedimentation analyses indicated that the composition of newly synthesized presynaptic RNA was essentially the same of the axon perfusate.

More recent experiments with rat brain slices incubated with [^{3}H]uridine demonstrated that a conspicuous fraction of newly synthesized RNA is recovered in the synaptosomal fraction where it displays a markedly more heterogeneous composition than in the squid upon sedimentation analysis.

Data indicate that axons and nerve endings contain local systems of gene expression based on the transfer of glial transcripts to their largely autonomous translation systems. These systems participate to the maintenance and plasticity of the corresponding neuronal domains. This novel perspective emphasizes the opportunity to extend investigations on the involvement of the presynaptic gene expression system under conditions of physiological or pathological plastic events.

T07-04A

Glia Open Access Database (GOAD): a web-tool to study glia phenotypes in health and disease (www.goad.education)

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Recently a remarkable number of genome-wide transcriptome profiles from pure populations of glia cells has come available. This results in an unprecedented amount of data that can be used to study glia cells in health and disease. We recently developed the Glia Open Access Database (GOAD, url:

www.goad.education), to make this data easily available. GOAD contains a collection of recently published datasets from microglia, astrocytes and oligodendrocytes. GOAD is web-based tool with three main features that enable exploring and analyzing of this data. First, Differential gene Expression analysis provides genes that are significantly up and down-regulated with the associated fold changes and multiple-testing corrected p-values between two conditions of interest. Several comparisons of interest can be done simultaneously and a Venn diagram is generated, depicting unique and common genes. Second, the Quantitative gene Expression analysis investigates which genes are expressed in specific cell types. Third, the search utility is able to find a gene of interest and depict its expression in all available expression data sets. In conclusion, GOAD is a comprehensive Gene Expression Encyclopedia of glia cells in health and disease.

Image

T07-05A

Role of glial NF-kB in a mouse model of Multiple Sclerosis

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Multiple Sclerosis (MS) is an inflammatory disease of the central nervous system (CNS) causing white matter damage and in the long run neuronal impairment leading to broad clinical symptoms with often severe consequences for affected patients. With the cause still being unknown, the underlying mechanisms are thought to be either a destruction driven by the immune system, or a failure of myelin-producing cells. Proposed causes include environmental factors but also genetic predisposition. In this regard, the transcription factor NF-kB has become of high interest due to its involvement in several neuronal (dys-)functions but also neuro-inflammatory processes. NF-kB consists of five subunits, all of which are proven to be essential for the peripheral immune reaction and therefore are candidate driving factors for the pathogenesis of autoimmune diseases, such as MS. Here, we focused on CNS-specific functions of the transcriptionally active subunits RelA (p65) and c-Rel of the classical signal transduction pathway either by Nestin-Cre mediated deletion in all neuro-ectodermal cell populations ($RelA^{CNS\text{-}KO}$; $c\text{-}Rel^{CNS\text{-}KO}$), or by CNP1-Cre mediated deletion specifically in oligodendrocytes (OGD) ($RelA^{OGD\text{-}KO}$; $c\text{-}Rel^{OGD\text{-}KO}$). Following induction of Experimental Autoimmune Encephalomyelitis (EAE) by immunization with MOG peptide, an animal model for MS, clinical symptoms and disease severity were substantially attenuated under inactivation of RelA as well as c-Rel in both neurons and macroglia (astrocytes and OGD). Interestingly, deletions in OGD alone presented similar protective results, thus underlining the importance of CNS-intrinsic components in the pathophysiology of MS. The higher expression of NF-kB in OGD adjacent to florid plaques observed in MS patients supports these data (Bonetti, Stegagno et al. 1999). Considering the clinical importance of neuronal and in particular glial NF-kB in inflammatory processes, we are currently elucidating the CNS lesion load and detailed composition of the lesion sites by using immunohistochemistry, ultra-structural electron microscopy, and murine MRI. With these methods, we aim at the specification of NF-kB in its role for structural and cellular alterations, and its contribution to the occurrence of histopathological MS subtypes (Lassmann et al. 2000).

T07-06A

The role of zinc finger transcription factor Zfp276 during glial development

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In vertebrates the transcription factor Sox10 plays essential roles during development of myelinating glia within the peripheral (PNS) and central nervous system (CNS). Loss or mutations of Sox10 in these cell types is a cause of severe dysmyelinating diseases in mouse and human. Despite its crucial role during myelination downstream targets and interaction partners of Sox10 in the nervous system are only partly known. Recent transcriptome and ChIP-Seq data identified the yet uncharacterized transcription factor Zfp276 as a potential Sox10 target. Further analysis of its expression pattern during mouse embryonic development showed a high overlap with Sox10 in differentiating oligodendrocytes and Schwann cells. Subsequent functional studies using a Zfp276 knockout mouse model and *in vitro* assays will help to elucidate the effector-target relationship between Sox10 and Zfp276 and to characterize the functions of Zfp276 as a mediator of Sox10-dependent regulation of glial differentiation and myelin maintenance.

T07-07A

Single-cell transcriptomics of the oligodendrocyte lineage in the mouse brain

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Oligodendrocytes (OL) are myelin-producing glial cells that insulate neurons. Defects in myelination are present in several diseases, such as multiple sclerosis (MS). The process of remyelination can be promoted by endogenous OL precursor cells (OPCs) at early stages of MS, ultimately failing as the disease evolve. OPC differentiation and myelination depends on several layers of transcription regulation driven by transcription factors (TF), chromatin-modifiers and non-coding RNAs (ncRNAs). Understanding the regulation of OL differentiation is essential to clarify the reasons behind the defective myelination in pathologies and open the pave to new remyelinating therapies. We are currently characterizing the transcriptome of cells of the OL lineage during development and in the adult mouse, to identify key transcriptional regulators involved in transition between epigenetic states in OPCs and OLs. For this purpose, we performed single cell RNA sequencing of 1000 cells from OL lineage obtained from cortex and hippocampus of adolescent mouse brain. In parallel, we performed single cell RNA-Seq in the OPC cell line Oli-neu upon differentiation in vitro. We identified several sub-populations of cells in oligodendrocyte lineage, some of which are novel and represent unique stages during the process of differentiation *in vivo*. We are currently characterizing these populations in the brain and spinal cord. We have identified ncRNAs and TFs that are uniquely expressed in different sub-populations of the oligodendrocyte lineage and we are currently analyzing the function of these novel candidates in OL development.

Support: Swedish Research Council, European Union (FP7 Marie Curie), Åke Wiberg Foundation, Karolinska Institutet research funds.

T07-08A

Sox2 beyond its stem cell role - New functions in oligodendroglial differentiation

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The transcription factor Sox2 is widely known for its role in the maintenance of progenitor characteristics in neural precursor cells (NPC). During development, not only neurons but also oligodendrocytes, the myelinating glia of the central nervous system, arise from NPC. We found the HMG-box transcription factor Sox2 and its close relative Sox3 continuously expressed in oligodendroglia into the stage of terminal differentiation. To elucidate the role of Sox2 and Sox3 in differentiation and myelination, we deleted them specifically in oligodendrocyte precursor cells (OPC). Therefore, we generated mice with a Sox10::Cre-mediated deletion of Sox2 and Sox3. In the absence of Sox2 and Sox3, myelin gene expression was reduced, whereas proliferation and colonization of the spinal cord with OPC was unaltered. The embryonic myelin deficiency was even more pronounced when both, Sox2 and Sox3, were deleted together pointing to a redundant function of those two transcription factors in CNS myelination. Mechanistically, we found a repressive effect of Sox2 on microRNA145 expression which itself inhibited expression of pro-myelination factors. Therefore, increased levels of microRNA145 due to the absence of Sox2 may contribute to the myelin deficiency in mutant mice.

T07-09A

Microglial transcriptome diversity in the healthy adult brain reveals regional heterogeneity in immunoregulatory and metabolic function and selective sensitivity to ageing

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An expanding array of homeostatic functions for microglia during brain development and the adult steady-state is emerging in addition to their established roles as key immune sentinels and effectors of the CNS. Heterogeneity in structure, cytoarchitecture and function across the CNS would be expected to place heterogenous demands on microglia. A better knowledge of microglial heterogeneity is needed to understand how microglia support normal brain function and may reveal region-specific sensitivities predisposing to age-related neurodegeneration. Here we show region-dependent microglial heterogeneity on a genome-wide scale and the functional pathways underlying adult steady-state microglial diversity.

Adult mouse microglia were purified from discrete brain regions and whole genome expression assessed by microarray. Principal components analysis revealed region-dependent heterogeneity in microglial transcriptomes and network analysis identified three major patterns of gene co-expression underpinning this heterogeneity. Transcriptional networks controlling bioenergetic and immunoregulatory function were the major processes responsible for microglial diversity. Differences in immunophenotype indicated a more immune vigilant state of cerebellar and hippocampal microglia but this phenotype was distinct from conventional states of activation. Functional differences in the ability of microglia from different brain regions to sequester bacteria and control replication correlated with the regional pattern of immune vigilance. Comparison with systemic macrophage transcriptomes showed that microglial regional diversity is superimposed upon a core profile distinguishing microglia from non-CNS macrophages. Regional differences in microglial heterogeneity are amplified during ageing and suggest region-dependent kinetics of microglial ageing.

T07-10A

Role of Inhibitor of DNA binding 4 (Id4) in adult neurogenesis

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The subventricular zone (SVZ) is one of the largest neurogenic niches in the adult brain of mammals. The SVZ harbours self-renewing neural stem cells (NSCs), which give rise to neural progenitor cells. These progenitor cells generate neuroblasts that migrate tangentially in chains to the olfactory bulb, where they differentiate into olfactory interneurons. Neurogenesis in the SVZ is tightly regulated by several intrinsic and extrinsic factors. Among extrinsic factors, the bone morphogenic protein (BMP) signalling pathway has been shown to positively regulate neurogenesis while inhibiting oligodendrocyte differentiation. However, the downstream transcriptional factors mediating the effects

of BMPs on adult neurogenesis are not well characterized. Inhibitor of DNA binding 4 (Id4) is a helix-loop-helix (HLH) transcriptional regulator activated by BMP signalling. Id proteins participate in numerous cellular processes such as differentiation, proliferation and apoptosis, by dimerizing with bHLH transcription factors, preventing them from binding DNA. Id4 is strongly expressed in the developing central nervous system and regulates neural progenitor proliferation and differentiation. However, Id4 function in adult neurogenesis is unknown.

In the present work, we sought to understand the function of Id4 in adult neurogenesis. We first determined the expression of Id4 protein in the adult SVZ by immunohistochemistry. We found that Id4 was expressed in stem cell astrocytes, neural progenitor cells and neuroblasts. To investigate Id4 role in the adult NSC niche, we analysed the cellular organization in the SVZ of *Id4-/-* mice. We found decreased numbers of proliferating cells and neuroblasts in the absence of Id4. As a result, there was a decrease in the proportion of newborn neurons in the olfactory bulb. Taken together, our results indicate that Id4 regulates neurogenesis from adult neural stem cells.

T07-11A

AAV-mediated gene therapy in dystrophin-Dp71 deficient mouse leads to blood-retinal barrier restoration

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Question: Dystrophin-Dp71 being a key membrane cytoskeletal protein, expressed mainly in Müller cells, its absence has been related to blood-retinal barrier (BRB) permeability through delocalization and down-regulation of the AQP4 and Kir4.1 channels (Sene, Plosone 2009). Dp71-null mouse is thus an excellent model to approach the study of retinal pathologies showing BRB permeability. The Adeno-Associated Virus (AAV) variant, ShH10, engineered to target glial cells specifically, has been characterized to transduce more efficiently Müller cells through intravitreal injection in the Dp71-null mouse (Vacca, Glia 2014). Here, we use ShH10 to restore Dp71 expression in Müller cells to study molecular and functional effects of this restoration in an adult mouse.

Methods: The GFP-2A-Dp71 sequence was cloned in the pTR-SB-smCBA to produce by triple transfection method, the recombinant AAV, ShH10-GFP-2A-Dp71. 1.8x10E10 particles of AAV was injected into the vitreous of 8-weeks-old Dp71-null mice. GFP expression was followed by fundus imaging and retinal thickness by OCT. Mice were sacrificed two months after injection. RNA and proteins were extracted to evaluate Dp71 and related proteins expression level. The localisation of these proteins was highlighted by immunocytochemistry on isolated Müller cells. The BRB permeability was evaluated by the Evans blue method.

Results: Fundus imaging revealed strong, pan-retinal expression of GFP fluorescent reporter, indicating robust transgene expression. Dp71 mRNA and protein were overexpressed (n=7) in the treated Dp71-null mouse as well as several Dp71 related proteins (β-dystroglycan, Kir4.1, AQP4). Moreover, the immunochemical analysis showed that exogenous Dp71 reaches a similar location than in WT mice (n=4). The OCT images revealed that PBS injection triggered a significant increase of the Dp71-null mice retinal thickness whereas Dp71 restoration leads to the the surgically-caused edema reabsorption (n=9). Interestingly, the BRB permeability as well as the VEGF level of Dp71-null mice was completely restored two months after ShH10-GFP-2A-Dp71 injection (n=10).

Conclusions: Here we clearly demonstrate that, in Dp71-null mouse with compromised BRB, we can restore a normal BRB permeability by restoring Dp71 expression via ShH10. This study is an important step forwards the development of new treatments of diseases with a BRB breakdown (AMD and diabetic retinopathy).

T07-12A

Increased Sox10 levels directly convert satellite Glia into oligodendrocyte-like cells in vivo

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Multiple Sclerosis is an inflammatory neurodegenerative disease of the central nervous system that leads to destruction of myelin sheaths and the myelin-forming oligodendrocytes. Therefore it is an important aim of current research to find ways of myelin regeneration, for instance by generating new oligodendrocytes through reprogramming or cell conversion strategies. Using a transgenic approach, we overexpressed transcription factors Sox10 or Olig2 throughout the peripheral nervous system in glial cells. We found that increased Sox10 expression induced oligodendrocyte transcription factors Olig2, Olig1, Nkx2.2 and Myrf in satellite glial cells of dorsal root ganglia, and led to ectopic expression of central myelin genes. An upstream enhancer mediated the direct induction of the Olig2 gene by Sox10. The oligodendrocyte-like cells from dorsal root ganglia furthermore extended multiple processes to axons and acquired a morphology characteristic of myelinating oligodendrocytes when co-cultured with neurons. Unlike Sox10, Olig2 was not capable of generating oligodendrocyte-like cells when overexpressed in dorsal root ganglia. Our findings provide proof-of-concept that even relative modest increases of Sox10 can convert susceptible cells into oligodendrocyte-like cells *in vivo.* They thus delineate options for Multiple Sclerosis therapeutic strategies.

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T07-13A

The role of FoxO3a in oligodendrocyte precursor cell differentiation

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The increasing effectiveness of new disease-modifying drugs that suppress disease activity in multiple sclerosis (MS) has opened up opportunities for regenerative medicines that enhance remyelination and potentially slow disease progression. A major cause for the failure of remyelination in MS is the blockage of oligodendrocyte lineage cell differentiation into myelinating oligodendrocytes. Understanding the molecular processes that regulate oligodendrocyte precursor cell (OPC) differentiation is therefore necessary to identify new treatment strategies for promoting remyelination in MS.

Cell development and maturation within the central nervous system is complicated and requires extensive interactions through external and internal regulators. Transcription factors in specific have been noted to play a crucial role in influencing oligodendrocyte precursor cell OPC differentiation. A microarray analysis of early regulators of OPC differentiation conducted in our laboratory identified FoxO3a, a transcription factor that is highly expressed in the brain, to significantly down regulate during the onset of OPC differentiation, and hence negatively regulate OPC differentiation. By blocking FoxO3a activity, we were able to promote OPC differentiation *in vitro* and enhance myelination *in vivo*.

Metabolic changes occurring during OPC differentiation have not yet been rigorously studied. Recent experiments we conducted indicate that the way OPCs cells generate energy changes radically when they differentiate into oligodendrocytes and start to form new myelin sheaths. Our preliminary experiments indicate that mitochondrial activity increases as OPCs differentiate. FoxO3a is a known regulator of mitochondrial activity and may control OPC differentiation. Our data suggests that

silencing FoxO3a, increases mitochondrial abundance and biogenesis. This demonstrates an important role of FoxO3a in the regulation of mitochondrial activity during OPC differentiation, and ongoing work is aimed at characterising the mechanisms through which this occurs.

Collectively, the results thus far makes FoxO3a an attractive candidate for promoting myelin repair. Furthermore, it may contribute to the identification of novel targets that can be used to promote central nervous system remyelination in Multiple Sclerosis.

T07-01B

Deciphering the role of *Etv5* in neural crest progenitor development and Schwann cell fate specification

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Schwann cells are the principal glial cells of the peripheral nervous system. Embryonically, Schwann cells originate from multipotent neural crest (NC) progenitor cells that give rise to Schwann cell precursors. These precursors develop into immature Schwann cells that then transition into a pro-myelinating state and eventually become myelinating Schwann cells. During these distinct developmental stages, cells in the Schwann cell lineage display distinct gene expression profiles. Multipotent NC progenitor cells express the Ets domain transcription factor *Etv5*, which is downregulated as the cells differentiate into Schwann cell precursors. Here we asked whether *Etv5* is required for the specification and differentiation of Schwann cells using a mutant approach. To examine the role of *Etv5*, we first characterized the development of NC progenitors and Schwann cells in wild-type embryos by examining the sequential onset of expression of markers that have been implicated in their development. A temporal expression profile was generated for developmental stages between E9 to P7, examining neural crest progenitor markers AP-2α, Etv5, Pax3, Snai1,Snai2, Sox9, Sox10, as well as markers implicated in later stages of Schwann cell development, such as Jun, NFATc4, Pou3f1,Pou3f2, Krox20, Krox24, Yy1, p75NTR, BFABP, GFAP and S100. To determine the function of *Etv5* in NC progenitor survival and glial specification, we examined the expression of these markers in hypomorphic *Etv5* mutant mice (*Etv5*^{tm1Kmm}), which are viable embryonically and postnatally. Analyses at E12.5 (Schwann cell precursor stage) revealed that *Etv5* mutants produce fewer glial cells in the dorsal root ganglia. We are currently examining embryos at later stages, the results of which will be reported. Taken together these studies will help us to elucidate the transcriptional cascades that underlie the earliest stages of SC lineage specification, and will help us to understand the intricate interplay between intrinsic and extrinsic signals. This information can be exploited in cellular reprogramming strategies aimed at generating a source of Schwann cells for glial support therapy.

T07-02B

Direct conversion of fibroblasts into functional astrocytes by defined transcription factors

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Direct cell reprogramming enables direct conversion of fibroblasts into functional neurons and oligodendrocytes using a minimal set of cell lineage-specific transcription factors. This approach is rapid and simple, generating the cell types of interest in one step. However, it remains unknown whether this technology can be applied to convert fibroblasts into astrocytes, the third neural lineage. Astrocytes play crucial roles in neuronal homeostasis and their dysfunctions contribute to the origin and progression of multiple human diseases. Herein, we carried out a screening using several transcription factors involved in defining the astroglial cell fate and identified NFIA, NFIB and SOX9 to be sufficient to convert with high efficiency embryonic and post-natal mouse fibroblasts into astrocytes (iAstrocytes). We proved both by gene expression profiling and functional tests that iAstrocytes are

comparable to native brain astrocytes. This protocol can be then employed to generate functional iAstrocytes for a wide range of experimental applications.

T07-03B

Brca1 is expressed in human microglia and is deregulated in human and animal model of ALS

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There is growing evidence that microglia are key players in the pathological process of amyotrophic lateral sclerosis (ALS). It is suggested that microglia have a dual role in motoneuron degeneration through the release of both neuroprotective and neurotoxic factors. To identify candidate genes that may be involved in ALS pathology we have analysed at early symptomatic age (P90), the molecular signature of microglia from the lumbar region of the spinal cord of $hSOD1^{G93A}$ mice, the most widely used animal model of ALS. We first identified unique $hSOD1^{G93A}$ microglia transcriptomic profile that, in addition to more classical processes such as chemotaxis and immune response, pointed toward the potential involvement of the tumour suppressor gene breast cancer susceptibility gene 1 (Brca1). Secondly, comparison with our previous data on $hSOD1^{G93A}$ motoneuron gene profile substantiated the putative contribution of Brca1 in ALS. Finally, we established that Brca1 protein is expressed in control human spinal microglia and is up-regulated in ALS patients.

Overall, our data provide new insights into the pathogenic concept of a non-cell-autonomous disease and the involvement of microglia in ALS. Importantly, the identification of Brca1 as a novel microglial marker and as potential contributor in both human and animal model of ALS may represent a valid therapeutic target. Moreover, it points toward novel research strategies such as investigating the role of oncogenic proteins in neurodegenerative diseases.

T07-04B

Rapid and highly efficient induction of oligodendrocytes from human pluripotent stem cells by forward programming

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Human pluripotent stem cells (hPSCs) represent a virtually unlimited source of human cells which, in theory, can give rise to all cells of the organism. However, for certain cell types that are of considerable interest for regenerative medicine, such as oligodendrocytes, robust and scalable differentiation protocols are still lacking. Reprogramming technologies based on overexpression of key transcription factors offer an alternative route for rapid and efficient generation of such cell types. We developed an improved method to conditionally overexpress transgenes in human pluripotent stem cells by using an optimized doxycycline-responsive inducible system. We applied this system to transiently overexpress key transcription factors of the oligodendrocyte lineage. By combining the forced expression of such master regulators of the oligodendrocyte lineage with environmental cues from developmental differentiation we derived a rapid and deterministic protocol for the induction of unlimited numbers of oligodendrocyte precursors within one week. Finally, we demonstrate that these cells efficiently differentiate into mature MBP-expressing oligodendrocytes.

T07-05B

Identification of a new potential marker for a subpopulation of astrocytes

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In the central nervous system (CNS), despite the predominant research attention on various neuron subtypes, astrocytes are dominant in number. Diverse morphology of astrocytes across CNS has been described, which is likely to reflect their molecular heterogeneity. Furthermore, astrocytes have been implicated in a wide variety of physiological activities such as synaptogenesis, neurotransmission and trophic regulation. However the molecular mechanisms that specify different subpopulations responsible for these diverse functions are still poorly understood. Using different histological techniques (e.g. *in situ* hybridization and immunohistochemistry) we have newly identified one potential specific marker, Gsx2 (a transcription factor), for a subtype of astrocytes. We will further investigate the functions of Gsx2 using a variety of approaches including conditional gene knockout in different regions of the brain and spinal cord, regional astrocyte ablation, electophysiological analysis and behavioural study to reveal its role in synaptic function and in the interaction between astrocytes and neurons.

T07-06B

Interactions of Sox10 with TGF-B SIGNALING in Schwann cells

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Schwann cell development is regulated by integration of intrinsic gene expression programs and external cues that affect Schwann cell proliferation and differentiation. One regulator of Schwann cell development is TGF beta signaling, which controls proliferation and apoptosis of embryonic Schwann cells, and Schwann cells themselves can also synthesize TGFbeta molecules. However, as Schwann cells mature, they become less responsive to TGF beta signaling. Sox10, an SRY-related HMG-box transcription factor, is crucial for embryonic development and ultimate differentiation of Schwann cells. While Sox10 has been shown to activate a number of molecules in Schwann cell development, we explored the possibility that Sox10 may also directly repress certain genes. Microarray analysis of Schwann Cells treated with an siRNA targeting Sox10 identified several genes that become induced. The induced genes were analyzed and found that several of the Sox10-repressed genes are involved in the TGF-B pathway. Reduction of Sox10 also lead to induction of a Smad 2/3-responsive (SBE) reporter plasmid, and activation of the reporter plasmid by TGFb1 is amplified when Sox10 is ablated. Furthermore qRT-PCR studies showed TGF-B target genes induced by Sox10 downregulation and this induction was further augmented under TGF-B ligand treatment. Lastly we have found that Dusp15, a phosphatase under the control of Sox10 represses the SBE reporter plasmid. Together, these data suggest that Sox10 represses the TGF-B pathway in SCs but not directly. Overall, these results suggest that Sox10 inhibits TGFbeta pathway activation in Schwann cells, with the possible mechanism being the induction of Dusp15.

T07-07B

Impact of transcription factor Sox13 on oligodendrocyte development in the embryonic mouse spinal cord

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Whereas the role of Sox5 and Sox6 transcription factors in the development of the vertebrate central nervous system have been intensely studied, little is known about the third closely related member of the SoxD family, Sox13. To fill this gap, we analysed Sox13 expression in the developing mouse spinal cord, and found it predominantly expressed in neuroepithelial precursors, oligodendroglial and astroglial cells. The substantially overlapping expression of Sox13 with Sox5 and Sox6 in

oligodendroglial cells prompted us to study potential roles during specification, lineage progression and differentiation of oligodendrocytes.

However, Sox13 deletion by itself did not interfere with any of these processes. Sox13 deficiency also did not aggravate the developmental defects triggered by loss of Sox6 in oligodendrocytes. This argues that Sox13 has no readily detectable major function in oligodendrocyte progenitors, and that oligodendrocyte development is primarily under control of Sox5 and Sox6. In contrast to Sox5 and Sox6, Sox13 expression continues after differentiation and even increases in myelinating oligodendrocytes. Therefore, a potential role on myelin maintenance will be the subject of further studies.

T07-08B

Astrocyte-specific transcriptional response to glucocorticoid receptor stimulation - metabolic implications

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Recent research indicates astrocytes are a major cellular target for glucocorticoid (GC) action in the central nervous system. Here we analyzed transcriptome of selective cell types originating from brains of mice treated with a glucocorticoid receptor (GR) agonist dexamethasone (Dex) *in vivo*. Astrocyte- and neuron-enriched cell populations were acutely isolated by magnetic cell sorting 4h after Dex (4 mg/kg i.p) administration. Microarray analysis (FDR 5%) revealed that neuronal response to Dex is limited to a strong upregulation of only a few specific transcripts, while GR activation *in vivo* modulates an array of GR-dependent genes in astrocytes, corroborating data on GR-related transcriptional profile *in vitro* observed by us previously. Among them are the serum/glucocorticoid -regulated kinase 1 (Sgk1) and pyruvate dehydrogenase kinase 4 (Pdk4) which regulates substrate influx into cytric acid cycle, implying GCs may alter astrocytic metabolic processes.

To investigate possible phenotypic consequences of GR-dependent signaling in astrocytes we utilized murine primary cell cultures to measure several parameters related to carbohydrate metabolism. Cell stimulation with 100 nM Dex for 24h resulted in an increased glucose uptake and decreased glycogen content that was associated with an enhanced production of lactate suggesting there may exist a GR-dependent metabolic switch increasing astrocytic trophic support for neurons in response to glucocorticoids. To determine the involvement of the GR-regulated genes in these effects we employed a lentiviral vector-based approach for RNA interference-mediated gene silencing *in vitro.* It allowed us to routinely achieve a 70-80% knockdown of basal mRNA expression and prevent target induction upon Dex stimulation over the level of unstimulated control. Sgk1 silencing abolished Dex-evoked increase in glucose uptake, possibly through regulation of glucose transporters' membrane localization, while there were no effects of Pdk4 knockdown.

Thus GCs appear to modify astrocytic glucose uptake via activation of Sgk1-dependent pathways. The glucocorticoid receptor-mediated modulation of astrocytic functions may contribute to mechanisms underlying response to stress and may be of relevance for stress-related brain disorders.

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T07-09B

Local self-renewing of microglia is dependent on Interleukin-1 signaling

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Microglia arise during early embryogenesis from yolk sac progenitors populating the developing CNS, where they are maintained as tissue-resident macrophages throughout the organism's lifespan.

Here, we describe an experimental system that allows the specific efficient conditional ablation of microglia *in vivo*. In a genetic approach we target microglia specifically in the CX_3CR1^{creER} x $iDTR^{ff}$ mice, and the administration of the Diphtheria toxin leads to microglia ablation with an efficienty of 80-90%.

Strikingly, we found that the microglia compartment was reconstituted within one week following depletion. In contrast to previous reports, microglia repopulation relied entirely on a CNS-resident internal pool that does not originate from bone marrow-derived precursors. In addition, the newly formed microglia are organized in highly proliferative micro-clusters that dissolve once steady state is achieved. These proliferating microglia in particular expressed the IL-1 receptor. Challenging animals during the repopulation phase with IL-1 receptor antagonist impaired microglia proliferation.

We therefore firstly conclude that, under physiological conditions, microglia self-maintain without the contribution of peripheral myeloid cells and secondly, that microglia proliferation depends on Interleukin-1 signaling.

T07-10B

Astrocytic CREB is a therapeutic target in acute brain injury

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The clinical challenge in acute injury as in traumatic brain injury (TBI) is to halt the delayed neuronal loss that happens hours and days after the insult. We report that the activation of the transcription factor cAMP-response element binding protein (CREB) in reactive astrocytes prevents secondary injury in an experimental model of TBI. The study was performed in a novel bitransgenic mouse wherein a constitutively active CREB, VP16-CREB, was targeted to astrocytes by the Tet-Off system. Using histochemistry, qPCR, and gene profiling we found less neuronal death and damage, reduced macrophage infiltration, and rescued expression of mitochondrial pathways in the bitransgenic mice as compared to wild type littermates. Finally, by meta-analyses using publicly available databases we identified a core set of VP16-CREB candidate target genes that may account for the neuroprotective effect. Enhancing CREB activity in astrocytes thus emerges as a novel avenue in acute brain post-injury therapeutics.

T07-11B

Development and validation of flexible system for selective genetic manipulation of astrocytes in wild-type mouse

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Increasing interest in astrocytes, as important regulators of brain physiology and pathology, calls for new tools to specifically alter signaling pathway in these cells. Current methods for gene manipulation (transgenic lines, viral vectors) prevent the screening of large numbers of genes due to their high cost and long timescales. To overcome this limitation, we have developed an approach enabling the expression of a transgene of interest in an astrocyte-specific fashion, on an otherwise wild-type background, in mice.

Our approach relies on the delivery of transgenes to glial progenitors in the mouse embryo. This goal is accomplished through intraventricular injection of a plasmid at embryonic day 16, followed by a set of electric pulses delivered through platinum electrodes, which are oriented to target cortical progenitors. Due to the intense proliferation of astrocytes in early post-natal development, the transgene needs to be incorporated into the genome to prevent plasmid dilution. This goal is achieved through the use of a transposon-based system, in which a self-inactivating transposase is encoded on the same plasmid as the transgene to be mobilized. Selective expression is achieved using an astrocyte-specific promoter.

To validate our system we injected a plasmid carrying the gene encoding the fluorescent protein TdTomato (under the control of an astrocyte-specific promoter) to the lateral ventricle of CD1 mouse embryos. Analysis of brains obtained at post-natal day 21 revealed expression of TdTomato in a large number of astrocytes in the targeted region of cortex.

A major advantage is its capacity to handle large transgenes. We have proven this by co-expressing two proteins (fluorophore and genetically encoded calcium indicator) separated with a P2A cleavage site from a single transgene. We are currently extending the system to enable Cre-dependent expression and gene knockdown.

Large transgenic capacity, low production cost and the flexibility to combine various genetic elements (promoters, transgenes etc) make this new system very promising for functional screens of neurodegenerative disease-related genes in astrocytes.

T07-12B

Definition of the microglial activome from individual mice revealed by RNAseq

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Microglial cells are the brain resident myeloid cells. Under physiological conditions they play important role in the surveillance and the control of the parenchyma homeostasis. Under pathological conditions microglia undergo phenotypic switch (or activation), a process that is shaped by the type and the intensity of the triggering signals. Recently, using genome wide analysis, studies have determined the transcriptome of microglial cells. Yet, these data were limited either to microglia under physiological

conditions or to specific microglia subtype, leaving open the question of microglial transcriptional remodeling underlying activation in pathological conditions.

In the present study, we used RNA sequencing to compare the quantitative transcriptome of microglial cells in both physiological (resting) and inflammatory (activated) states triggered by systemic LPS administration, a paradigm known to promote microglial activation and progressive neurodegeneration. More specifically, we took advantage of CX3CR1$^{+/gfp}$ mice, in which microglia are the only brain cells to express GFP, to purify cortical microglial cells by FACS. This allowed us to compared the repertoire of genes expressed by microglia isolated from individual mice under physiological and inflammatory condition

More than 10 000 genes were identified in both quiescent and activated microglia. The activation paradigm induced the deregulation of about 2000 genes. This list of genes was further refined to a core of 371 genes that were selected according to specific criteria including (1) magnitude of deregulation; (2) expression level and (3) cell expression specify. Based on these selected genes we constructed a TLDA array and investigated the changes in microglial gene expression in the hippocampus after induction of experimental Status Epilepticus.

On a technical point of view, our results show that combination of microgenomic and RNAseq approaches can be used to define the transcriptome of microglial cells isolated from individual mice. These approaches lead us to the characterization of the molecular signature of in-vivo activated microglial cells. We also identified a subset of 150 genes that are deregulated in at least two independent CNS pathological conditions, and that defines the core of microglial activome. This activome will represent a useful tool to unambiguously investigate microglial activation in different physiological and pathological conditions.

T07-13B

Targeting microglia using the specific transcription factor Sall1

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Microglia are the resident macrophage population of the central nervous system (CNS). In contrast to most other populations of the mononuclear phagocyte system, adult microglia derive from primitive myeloid progenitors that arise in the yolk sac early during embryonic development. In the steady state, microglia maintain themselves locally and independently of circulating bone marrow derived precursors.

By comparing the transcriptome of microglia to other tissue macrophage populations we identified *Sall1*, a gene which codes for a transcription factor and represents a promising candidate to achieve microglia-specific targeting. We could show that of all hematopoietic cells, *Sall1* is expressed exclusively by microglia. During embryogenesis, Sall1 is not expressed on primitive yolk sac macrophages but microglia precursors in the developing CNS start to express Sall1 from E14.5 and continue to express it throughout life. Even though *Sall1* is highly expressed by all microglia, we demonstrated that Sall1 is neither crucial for microglia development and homeostasis nor for their activation in neuro-inflammation. Additionally myeloid cells infiltrating the CNS during neuro-inflammatory conditions do no acquire Sall1 expression indicating that Sall1 expression is unique to microglia and that *Sall1*Cre^{ER} mice can be utilized for microglia specific gene-targeting without undesirable targeting of other myeloid cells.

Poster topic 08
Glial-neuronal interactions

T08-01A

Role of astroglia (pituicytes) in the hypothalamo-neurohypophyseal system - a major brain-to-blood neuro-endocrine interface

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The hypothalamo-neurohypophyseal system (HNS) is an interface through which the brain regulates body homeostasis by means of releasing the hypothalamic neurohormones oxytocin and arginine-vasopressin to the general circulation. The basic components of the HNS are the hypothalamic axonal projections, endothelial blood vessels and astroglial-like cells, termed pituicytes. These three tissue types converge and interact at the ventral forebrain to establish an efficient neuro-vascular interface, which allows the release of neurohormones from the brain to the periphery. However, the mechanism underlying this process and in particular the role of the pituicytes is unknown. In the adult animal, pituicytes may facilitate hormone secretion from neurohypophyseal axons to the perivascular space but their exact role is still not clear[1]. Using zebrafish as model organism, our aim is to study the role of pituicytes in HNS development and function. Towards this goal we have been (i) developing new tools to label, image and isolate the pituicytes (ii) identifying pituicytes molecular signature (iii) employing genetic tools to explore the role(s) of candidate molecules that could mediate interaction between pituicytes and axo-vasal structures. Our systematic cellular and molecular characterization of HNS astroglia is expected to shed light on the role of pituicytes in a vital neuroendocrine system in vertebrate.

Reference:

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T08-02A

Neuron and glia interaction regulates $GABA_A$ receptor expression in the oligodendrocyte membrane

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Axon myelination is the main function of oligodendrocytes (OLG) in the central nervous system. This highly concerted phenomenon requires of glia-neuron communication, and both chemical transmitters and contact interactions are essential for myelination. OLG are endowed with neurotransmitter receptors whose levels and properties vary during maturation and myelination. However, knowledge about how OLG-neuron interactions regulate those changes is scant. Here, we studied the expression and function of neurotransmitter receptors in oligodendrocyte progenitors (OPC) and OLG from the optic nerve in cells cultured alone or in the presence of dorsal root ganglion (DRG) neurons. Cell responses to GABA, glutamate, and ATP were recorded by patch-clamp at different days in vitro. Both OPC and OLG cultured alone in differentiation medium showed inward currents to these transmitters. Intriguingly, sensitivity to GABA drastically diminished to less than 10% in both cell types after 2 days in culture, while that of glutamate and ATP remained constant. In contrast, the amplitude of the GABA responses was unalterably high in either OPC or OLG co-cultured with DRG neurons. Immunochemistry and electrical properties indicated that OLG that were responsive to GABA in co-cultures with neurons were engaged in axon myelination, whereas OLG in the same culture but without axonal contact lost GABA sensitivity. Response was mediated by $GABA_A$ receptors (likely

formed by α3, β2/3 and γ1/3 subunits) with a distinctive pharmacology, and its activation elicited an increase in intracellular Ca^{2+} concentration, according with previous studies. The results strongly suggested that GABA receptor regulation may be relevant to OLG maturation and to OLG-neuron signalling.

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T08-03A

Astrocytic activity controls neuronal excitability upon brain ischemia

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Astrocyte shows dynamic activity in the brain, but the impact of this activity on neuron has not been well understood. Our previous research showed that astrocytic stimulation by channelrhodopsin-2 (ChR2) induces glutamate release and this glutamate modulates neuronal activity. Here, we further investigated the mechanism of its release. Unlike neuronal glutamate release, ChR2-photoactivated astrocytic glutamate release was calcium-independent. We thus searched for the actual trigger of astrocytic glutamate release. It is known that ChR2 has much more permeability to H^+ than Na^+ or Ca^{2+}. We found that lowering the buffering capacity of the intracellular pH induced stronger intracellular acidification in astrocytes in response to ChR2-photoactivation and this stronger acidification caused larger amount of glutamate release from astrocytes. This result suggests that acidification is a trigger for astrocytic glutamate release. Next, we investigated astrocytic contribution to excitotoxicity because the astrocytic activity that we found would likely operate maximally under pathological conditions. Brain ischemia is a situation that occurs upon cessation of blood supply to the brain. Two major events occur; acidosis and excess glutamate release. Although it is known that excess glutamate leads to neuronal cell death, cellular source of the glutamate and its release mechanism are not fully identified. Here we hypothesized that astrocytic severe acidification triggers excess glutamate release. To investigate this possibility, astrocytic acidification was countered with light stimulation of astrocytic archaerhodopsin (ArchT), a light sensitive proton pump. ArchT-photoactivation dramatically reduced astrocytic glutamate release and strongly inhibited ischemic brain damage. These results suggest that pH-dependent astrocytic glutamate release is one of the major cause ofexcitotoxicity upon brain ischemia. It would be important to study whether astrocytes regulate neuronal excitability in various brain conditions.

T08-04A

Analysis of purinergic P2Y1 receptors in cortical astrocytes and cerebellar Bergmann glia

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Astrocytes express a plethora of neurotransmitter receptors that form “microdomains” for signaling pathways along their processes. The purinergic P2Y1 receptor is involved in long-range intercellular signaling of astrocytes involving gliotransmitter release. In neuropathological situations, blocking of P2Y1 receptors can provide neuroprotection against glutamatergic exitotoxicity as occurring in stroke models.

To investigate and temporally control astroglial purinergic P2Y1 receptors more specifically in cerebellar Bergmann glia as well as in cortical astrocytes, we crossbred floxed P2Y1 mice with astrocyte-specific, tamoxifen-inducible Cre DNA recombinase (GLAST-CreERT2) mice. Successful recombination was determined by quantitative real time PCR (qRT-PCR) of genomic DNA using primers across the loxP sequences flanking exon 1 of *p2ry1*. DNA recombination was induced in adolescent mice by tamoxifen injection for three consecutive days and analyzed three weeks later. To reduce contaminating neuronal P2Y1 expression, we also studied FACsorted astrocytes, taking advantage of either endogenous reporter expression or specific antibody labeling. Structural changes of astrocyte morphology at synapses and vasculature was investigated by light- and electron microscopy. Astrocyte synapse contact number and length was quantified by stereological analysis

using electron microscopy. A major focus of this project addresses the characterization and comparison of spontaneous or behavior-evoked Ca^{2+} signals in awake control and receptor KO mice. For that purpose, the Ca^{2+} indicator GCaMP3 was additionally included into the conditional P2Y1 receptor mice. The imaging analysis will be complemented by behavioral analysis of motor behavior.

T08-05A

Fractalkine-receptor knock-out mice show unaffected depressive-like behavior and reduced microglia hyper-ramification after chronic-stress exposure

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Introduction: Microglia are suggested to be involved in several neuropsychiatric diseases, like depression. It is known that LPS injection in rodents induces depressive-like behavior and microglial pro-inflammatory activation, with processes retraction and acquisition of rounded shape morphology. Microglia and neurons closely interact through the CX3C axis. CX3CR1 (fractalkine receptor) is prominently expressed by microglia, while synthesis of its ligand CX3CL1/fractalkine is restricted to neurons. More recently it is has been found that in CXCR1 deficient animals LPS injection leads to enhanced microglia activation and longer persistence of depressive-like behavior. These data point towards a role of microglia in inflammation related depressive-like behavior. Pronounced depressive-like behavior can also be induced in mice after exposure to chronic-stress. Of note, in mice exposed to chronic-stress microglia do not exhibit pro-inflammatory phenotype, but appear surprisingly hyper-ramified. These different microglial responses suggest diverse microglia roles in distinct models of depressive-like behavior (i.e. induced by LPS or chronic-stress). We therefore aimed to clarify the role of microglia and CX3CR1 in a mouse model of chronic-stress.

Methods: In the present study we used $CX3CR1^{GFP}$ mice and investigated development of depressive-like behavior after repeated swim stress (10 min in 5 consecutive days). Depressive-like behavior was measured by forced swim test (FST) and tail suspension test (TST). Microglial morphology 4 weeks after the repeated swim stress was assessed by means of confocal microscopy and 3D cell reconstruction.

Results: In wild-type mice we observed the development of depressive-like behavior within 5 days. This depressive-like state was unchanged for the following 4 weeks, proving a chronically persistent stress-induced behavioral despair. Measurement of microglia 3D morphology revealed a robust transition towards microglia hyper-ramification. In $CX3CR1^{GFP}$ mice a clear resistance to stress-induced depressive-like behavior was observed. Furthermore microglia did not show signs of hyper-ramification under CX3CR1 knockout condition.

Conclusion: Our findings suggest that microglial CX3CR1 exerts opposite functions in different mouse models of depressive-like behavior. The biological consequences of microglia hyper-ramification are currently unknown. Further investigations are required to assess whether and how this morphological transition is linked with depressive symptoms.

T08-06A

D-serine acting on raphe nucleus and ventral respiratory column can mediate respiratory responses induced by hypercapnia in neonatal mice

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Question: Astrocytes are able to sense neuronal activity, react to, and in turn, influence the activity of neuronal circuits. Astrocytes in the retrotrapezoid nucleus (RTN) contribute to the modulation of the respiratory rhythm. Here, they sense changes in H^+ or PCO_2, and release ATP which excites the respiratory network. However, in other chemosensory nuclei like the raphe nucleus (RN), the nucleus tractus solitarius (NTS) and the ventral respiratory column (VRC) this role of ATP is uncertain. We reported that D-serine, an endogenous co-agonist for NMDAr and a known gliotransmitter in glutamatergic synapses, can modulate fictive respiration in brainstem slices from mouse neonates. Our aims were: 1) To study whether D-serine may contribute to the hypercapnia-induced modulation of the respiratory rhythm; and 2) To define sites of D-serine action by locally applying D-serine on different brainstem nuclei.

Methods: Fictive respiration was recorded from the VRC with glass suction electrodes in medullary slices obtained from neonatal CF1 mice (P0-P4). Superfusion was done with artificial cerebrospinal fluid (aCSF) equilibrated with O_2:CO_2 = 95%: 5%, (pH 7.4, 29 ±1°C) or during hypercarbia gassing aCSF with O_2:CO_2 = 90%: 10%, (pH 7.2). D-serine levels were reduced by enzymatic degradation with D-amino acid oxidase (DAAO) or by inhibiting its synthesis with D-serine racemase blockers. A pulse of D-serine 100 µM or vehicle (aCSF) were pressure ejected (3 psi, 10 s) into the brainstem nuclei using a borosilicate glass capillary (tip diameter of 3.75 µm) connected to a pneumatic picopump. D-serine levels in the medium were detected by HPLC.

Results: Hypercapnia induced the release of D-serine from brainstem slices. Application of DAAO or D-serine racemase blockers reduced the increase in the frequency of fictive respiration induced by D-serine application or hypercapnia. D-serine applied in RN and VRC, but not in NTS, increased the frequency of fictive respiration up to 127% of basal frequency (n=5) and up to 131% (n=4), respectively. Microinjection with vehicle did not alter the basal frequency of fictive respiration.

Conclusions: D-serine can increase the frequency of fictive respiration acting directly on VRG or RN. Since both nuclei are chemosensitive, these results support the idea that D-serine may be a mediator of the respiratory response to hypercarbia.

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T08-07A

Neuronal ndrg4 is essential for Nodes of Ranvier organization and myelination in zebrafish

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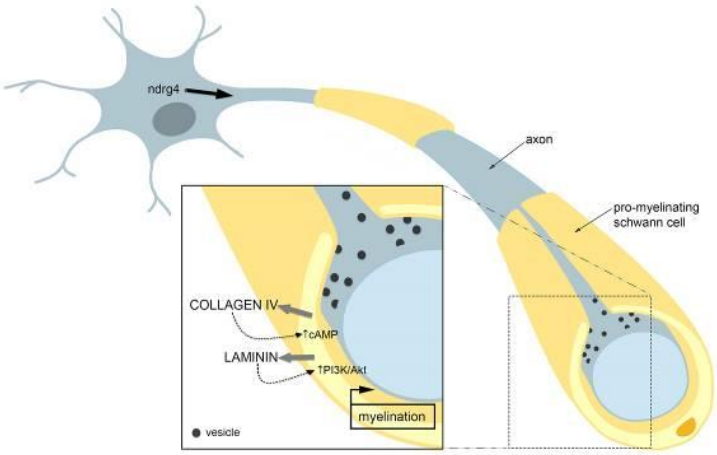
Axon ensheathment by specialized glial cells is an important process for a fast propagation of action potentials along the axons. However, little is known about direct axon-glia cross talk that ensures a proper myelination. We have identified the N-myc downstream-regulated gene, ndrg4, as a novel neuronal factor essential for myelination in zebrafish. A specific knockdown of ndrg4 leads to a loss of mbp and krox20 expression in the Peripheral Nervous System (PNS) while axonal growth and Schwann cells (SCs) development is intact. This coincides with a disruption in sodium channel clustering. Analysis of chimeric embryos show an autonomous requirement for ndrg4 in neurons for sodium channels clustering along the axons. Molecular analysis show that the expression of the t-SNARE protein, SNAP25 is, among others, sharply decreased, revealing a novel role of ndrg4 in controlling vesicle membrane fusion during exocytosis while vesicle transport, *per se*, along the axons is not affected. This is synchronized with a significant decrease in the expression of several Extra Cellular Matrix (ECM) components that are expressed and secreted by SCs e.g. Collagen IV and Laminin. Indeed, our results show that a specific knockdown of Collagen IV, *in vivo,* leads to peripheral myelin defects. Moreover, injection of Tetanus Toxin (TetTx) in zebrafish embryos, blocking synaptic vesicle release, in the whole nervous system does not lead to myelination defects in the PNS. Overall, our results reveal a previously unknown function for ndrg4 in modulating vesicle release that is essential for axon-glia interaction and therefore myelination and that is independent of its function in synaptic vesicle fusion, at least in the PNS.

Figure legend:

A schematic view of a potential neuronal ndrg4 function in PNS myelination.Neuronal ndrg4 controls axonal vesicle release, most likely towards SCs, by regulating a range of proteins required for exocytosis e.g. SNAP25. This vesicle exchange allows SCs to synthetize/secrete ECM components (grey arrows) such as LAMININ and COLLAGEN IV. Secreted LAMININ and COLLAGEN IV feedback to SCs (dotted arrows) activate PI3K/Akt pathway and increase cAMP levels respectively, an important step for myelination to proceed.

Image



T08-08A

Astroglial-mediated remodeling of the interhemispheric midline is exclusive to eutherian mammals and underlies the formation of the corpus callosum

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The corpus callosum forms the primary connection between the cortical hemispheres in the brains of eutherian (or placental) mammals. Congenital absence (or agenesis) of this major fiber tract is a common neurodevelopmental disorder that affects neurological function, yet the etiology underlying this disorder is unknown. We identify that a key process required for midline crossing of callosal axons is the remodeling and degradation of the intervening interhemispheric fissure during development. *In vivo* gain- and loss-of-function experiments in mice show that Fgf signaling to downstream Nfi transcription factors initiates this process by promoting the transition of radial glia into multipolar astroglia. These astroglia then intercalate with one another and degrade the intervening fibroblast tissue to provide a permissive substrate for callosal axon navigation. Neuroimaging studies reveal that defects in this process are a predominant cause of human callosal agenesis. Furthermore, comparative analyses show that remodeling of the interhemispheric fissure does not occur in acallosal mammals, such as marsupials and monotremes, strongly suggesting that this glial-mediated process is associated with the phylogenetic origin of the corpus callosum.

T08-09A

Glutamatergic astrocyte-neuron signaling is disrupted in Fragile X Syndrome

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Fragile X Syndrome (FXS), the most common cause of inherited human mental retardation and autism, is caused by a trinucleotide (CGG) repeat expansion in the X-linkled Fmr1 gene that results in the loss of the fragile X mental retardation protein (FMRP), a RNA binding protein regulating translation. Brain development in the absence of FMRP gives rise to a broad spectrum of psychiatric and neurological problems. The search for an effective treatment has focused primarily on gaining insights into the biological functions of FMRP on neurons and has repeatedly failed when translated to clinical trials in humans. For years, FMRP expression in the central nervous system was thought to be mainly neuronal. However, recent evidences demonstrated the expression of FMRP in astrocytes and other glial cells at developmental stages. The presence of FMRP in astrocytes during development seems essential to their role in synaptogenesis, since its loss results in abnormal dendritic spine morphology and immature looking synapses, a feature broadly described in FXS patients.

Astrocytes have emerged as active regulatory elements directly involved in synaptic physiology. They sense and integrate synaptic activity by responding with intracellular Ca2+ elevations to synaptically released neurotransmitters. This Ca2+ signal stimulates the release of gliotransmitters that regulate neuronal excitability and synaptic transmission and plasticity, suggesting an active role of astrocytes in brain function. For this reason, we investigated the properties of the astrocyte-neuron signaling and astrocyte-dependent modulation of synaptic transmission in hippocampal slices of mice lacking FMRP.

Our results show that glutamatergic activation of astrocytes is disrupted in Fmr1 knockout mice, whereas the cholinergic activation is intact, suggesting that astrocytic responsiveness to glutamatergic transmission is selectively impaired in mice lacking astrocytic FMRP. As a result, glutamate-induced gliotransmitter release is absent in Fmr1 knockout mice, which is associated with the loss of astrocyte-dependent long-term potentiation (LTP). This LTP can be rescued by exogenous application of the gliotransmitter D-serine.

We conclude that glutamatergic astrocyte-neuron signaling and astrocyte-induced synaptic plasticity phenomena are impaired in Fmr1 knockout mice, suggesting that disturbances of the astrocyte signaling also contribute to the Fragile X Syndrome and postulating astrocytes as potential drug targets in this syndrome.

T08-10A

VAMP expression in healthy and gliotic murine Müller glia cells

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Question: Currently, very little is known about the retinal expression of vesicle associated membrane proteins (VAMP) except for VAMP1 and VAMP2. Here, we investigated the expression pattern of known VAMPs with a special focus on retinal Müller cells as important prerequisite for further studies of VAMP functions in glial cells.

Methods: The distribution of different VAMPs was investigated by immunohistochemical staining in healthy and postischemic retinae. To explore cell-specific expression patterns, retinal glia and neurons were separated by magnetic activated cell sorting (MACS) and VAMP expression was compared at the transcriptional and translational level using qRT-PCR and Western blots. Immuno-electron microscopy was performed to analyse the subcellular localization of selected VAMPs in retinae.

Results: Immunohistochemical staining of retinal slices showed that VAMP1, VAMP2 and VAMP4 are primarily localized in retinal neurons. VAMP2 was located predominantly in synaptic layers and VAMP4 was mainly associated with the trans-Golgi network in retinal ganglion and amacrine cells. In contrast, VAMP3, VAMP5 and VAMP8 were strongly expressed by Müller glia. VAMP7 co-localized with the microglia marker Iba-1. These findings were verified at the transcript and protein level in isolated Müller cells. Immuno-EM demonstrated a high density of VAMP3 in Müller cell endfeet. The expression of all glial VAMPs was generally upregulated in gliotic Müller glia seven days after induction of ischemia.

Conclusion: Our analysis reveals that Müller cells express a distinct set of VAMPs, which changes under pathologic conditions. Future analysis will help to elucidate the functional role of distinct VAMPs in glial cells.

T08-11A

On the role of Ca^{2+} in apoptosis and necrosis of distant glial cells surrounding the axotomized sensory neuron in the crayfish stretch receptor

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Question: Both neurons and satellite glial cells support survival of each other. On the contrary, neuron injury can lead to either death or proliferation (reactive gliosis) of surrounding glial cells. Here we demonstrate that axotomy of the sensory neuron in the crayfish stretch receptor (CSR) results in necrosis and apoptosis of distant glial cells surrounding soma and proximal part of axon, and Ca^{2+} mediates glia death.

Methods: We developed the method of CSR isolation without axotomy that saves the axon connection to the abdominal ganglion. This intact neuroglial preparation (Int) served as control in comparison with the axotomized preparation (AT).

Results: Axotomy increased both necrosis and apoptosis of glial cells. Glial necrosis became significant at 15 h after isolation. In AT preparation it was higher than in the intact one. Glial apoptosis appeared earlier, 4-8 h after isolation. In intact preparations apoptosis level was not changed following 8 h incubation. In AT preparation it increased gradually from 4 to 15 h post-isolation and was higher comparing with Int preparation. 3-fold Ca^{2+} concentration in saline increased glial apoptosis in AT but not in intact preparations. 3-fold $[Ca^{2+}]o$ decrease did not influence glial apoptosis. Calcium ionophore ionomycin induced 2.7 and 6-fold increase in glial apoptosis around intact and axotomized neurons, respectively. 2.6-fold increase in the level of glial necrosis was significant only in AT preparations. Unexpectedly, glial necrosis in both in AT and Int preparations was decreased in the presence of 3 $[Ca2^{+}]o$, but increased in 1/3 $[Ca^{2+}]o$.

Conclusions: Thus, extracellular Ca^{2+} stimulated apoptosis of glial cells around the axotomized neurons but suppressed necrosis in both AT and Int preparations. The data on the role of Ca^{2+} in glial necrosis are still contradictory.

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T08-12A

Boosting astrocyte-neuron signaling by optical tools

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Astrocyte signaling has critical impact on brain physiology by releasing neuroactive substances, so-called gliotransmitters (Araque et al., 2014). In the last years, optogenetic has provided powerful approaches to non-invasively activate and control neuronal circuits. Here, we manipulate astrocytes with optogenetic tools to control their activity and evaluate their consequences on neuronal physiology. The ectopic expression of channelrhodopsin-2 (ChR2, a light-activated ion channel protein) was targeted specifically to astrocytes by viral transfection (Perea et al., 2014). Using electrophysiological techniques in brain slices, we found that optical activation of astrocytes enhanced local excitatory synaptic transmission in CA1 hippocampal pyramidal neurons and layer 2/3 neurons of somatosensory cortex. ChR2-stimulated astrocytes induced sustained potentiation of evoked synaptic responses associated with the duration of light stimulation. Neuronal activity to light stimulation was recorded in control slices and no significant responses were observed. The pharmacological analysis

indicated that astrocyte-induced modulation of synaptic transmission was mediated by activation of metabotropic glutamate receptors at neuronal membranes. Then, optical activation of astrocytes stimulates glutamate release that controls the synaptic strength of cortical neurons influencing the operation of particular cortical networks.

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T08-13A

Investigation of a glial-specific G-protein-coupled receptor

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There is growing interest in the normal functions of glia and their potential roles in neurological diseases. It has long been known that oligodendrocytes (OLs) and their precursors (OPs, also known as NG2 glia) are critically involved in demyelinating diseases such as multiple sclerosis. More recently, astrocytes have been recognized as having essential physiological functions in the normal CNS and have also been implicated in stroke, brain trauma, Alzheimer's disease, Parkinson's disease, motor neuron disease and Rett syndrome. The superfamily of G-protein-coupled receptors (GPCR) is a large family of seven-pass transmembrane proteins. The majority of these bind a specific extracellular ligand, which triggers an intracellular response. GPCRs are major drug targets; it has been estimated that more than half of clinically useful drugs interact with these receptors. We conducted a visual screen of expression patterns in the Allen Brain Atlas for gene products that seemed likely to mark glial cells and analysed these further by in situ hybridization and immunocytochemistry. Among several genes picked in this way, we found one GPCR (GPRX) that turns out to be expressed exclusively in glia. GPRX is first expressed at early postnatal stages (P7-P14), coinciding with the onset of synaptogenesis, and maintains its expression into adulthood. It is expressed throughout the adult brain and spinal cord in around half of all astrocytes and a similar fraction of OPs. Electrophysiological examination of hippocampal CA3-CA1 transmission in germline knockout mice showed that the stimulation strength needed to evoke a half-maximal field EPSP was reduced in the knockout, suggesting that GPRX modulates neuronal excitability or synaptic transmission, either through indirect effects on neurons or by influencing the extrasynaptic environment.

T08-14A

Astrocytes limit epileptiform discharge duration and restrict neuronal sodium loads

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During normal as well as pathological brain activity, changing ion concentrations induce and shape neuronal discharges. A major role herein can be attributed to sodium fluctuations experienced by both neurons and astrocytes. Influx of sodium into neurons must be limited to the minimum level required for electrical signaling because extrusion of sodium by the Na^+/K^+-ATPase is the most energy-expensive process in the brain. Astrocytes have been proposed to metabolically support neurons especially during phases of high energy demand. Astrocytes might thereby restrict activity related sodium influx into neurons and, in addition, dampen neuronal excitation via control of transmitter and ion homeostasis.

Here, we performed sodium imaging in mouse hippocampal slices combined with field potential and patch-clamp recordings and measurement of extracellular potassium ($[K^+]_o$). Network activity was induced by Mg^{2+}-free, bicuculline-containing saline. Neurons showed recurring epileptiform bursting and sodium increases by up to 22 mM, accompanied by transient increases in $[K^+]_o$ and astrocyte depolarizations. Astrocyte sodium concentration increased by up to 8.5 mM, which could be followed by an undershoot below baseline. Sodium oscillations required action potentials and activation of ionotropic glutamate receptors. Inhibition of glutamate uptake caused an increased discharge frequency, followed by cessation of electrical activity, irreversible sodium increases and swelling of neurons. The gliotoxin NaFAc (sodium-fluoroacetate) resulted in elevation of the sodium concentration in astrocytes and neurons and impaired glial uptake of glutamate and potassium. Moreover, NaFAc dramatically prolonged epileptiform discharges and accompanying sodium signals especially in neurons, most likely due to the decreased removal of glutamate and potassium by astrocytes.

Our study thus establishes that recurrent discharges result in sodium signaling in neurons and astrocytes and confirm the essential role of glutamate transporters for maintenance of network activity. They suggest that astrocytes restrict discharge duration and show that inhibition of glial metabolism impedes the neurons' capacity to maintain low intracellular sodium and to recover from activity-induced sodium loads, uncovering a hitherto unknown aspect of neuron-glia interaction at excitatory synapses. This interaction will especially be critical during periods of high activity to restrict sodium influx into neurons, preventing cell swelling and excitotoxic damage.

T08-15A

Lipopolysaccharide stimulated microglia up-regulate Na^+current density in cultured hippocampal neurons

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Microglial cells are involved in immune reactions of the brain. Various cytokines are released out of microglia upon stimulation by factors such as lipopolysaccharides (LPS) from bacterial membranes. Since inflammatory reactions in the brain may lead to neuronal hyperactivity such as epileptic seizures, which occurred in 20% of the patients in a recent infection by the O104:H4 *E.coli* strain (Magnus et al., 2012) we studied whether the addition of activated microglia effects the Na^+ current density (I_{NavD}) in cultured hippocampal neurons.

Preliminary investigations using whole-cell patch-clamp recordings in bipolar and pyramid-shaped neurons showed that the addition of 5% microglia stimulated by 1 µg/ml LPS resulted in a significant up-regulation of I_{NavD} in pyramid-shaped neurons and a smaller up-regulation in bipolar cells. Since tumor necrosis factor-alpha (TNF-α) is a key proinflammatory cytokine, that has been shown to up-regulate I_{Nav} in peripheral neurons (Jin and Gereau, 2006) we tested whether the effect of the addition of stimulated microglial cells can be mimicked by a preincubation of the cells with TNF-α concentrations between 10 and 100 ng/mL for 4 days. After this treatment I_{NavD} was significantly increased in pyramid-shaped neurons whereas smaller increases were observed in bipolar neurons. We now investigated, whether TNF-α is a dominant factor involved in the regulation of the I_{NavD} in cells cultured in the presence of activated microglia, using antibodies against the p55 and p75 TNF receptors. After co-cultivation of the cultures with 10 µg/ml of the antibodies the I_{NavD} regulation by LPS stimulated microglia was blocked in pyramid-shaped cells using a combination of both antibodies as well as the anti-p75-R but not the anti-p55-R. This suggests that activated microglia increase the I_{NavD} in hippocampal pyramid shaped neurons primarily via the p75 receptor. Since I_{NavD} in the mostly glutamatergic pyramid shaped neurons was regulated by a larger extent than in the mostly GABAergic bipolar neurons, TNF-α released from activated microglia might change the balance between excitation and inhibition. This could contribute to the generation of epileptic seizures in inflammatory conditions.

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T08-16A

Glial phagocytosis of apoptotic neurons in developing and mature CNS

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In the developing and mature central nervous system (CNS), a large number of neurons die through apoptosis and are efficiently removed by phagocytic glia. The precise removal of apoptotic neurons is crucial for the formation and maintenance of a functional CNS and must be tightly regulated. Despite its great biological importance, the molecular mechanisms underlying the relationship between neuronal apoptosis and glial phagocytosis remain unclear. Since apoptosis and apoptotic cell clearance are highly conserved in evolution, we use *Drosophila Melanogaster* as a model system, which provides powerful tools to study the establishment and function of glia as primary phagocytes in the developing and adult CNS.

We have recently demonstrated that glial ability to phagocytose apoptotic neurons is developmentally programmed and does not depend on apoptosis per se. In addition, we discovered a novel mechanism that is able to synchronize excess neuronal cell death and glial phagocytosis of dying neurons, which involves c-Jun N-terminal kinase (JNK) signaling. We demonstrated that JNK pathway gain-of-function in neurons leads to JNK signaling in glia, which results in upregulation of glial phagocytosis. The proposed mechanism may be important for removal of damaged neurons in the developing and mature CNS.

We also study the role of glial phagocytosis in neurodegeneration where we have discovered that glia overexpressing the phagocytic receptor SIMU cause neuronal loss in the adult brain accompanied by motor dysfunction and shorter life span of the affected flies. This study proposes a novel mechanism of neuronal loss, where the brain pathology originates from abnormal phagocytic function of glial cells.

T08-17A

Glutamate-induced astrocytic $[Na^+]_i$ elevation - a mechanism to increase K^+ clearance via the Na^+/K^+-ATPase?

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Neuronal activity in the brain is associated with transient increases in extracellular glutamate and K^+ concentrations. The surrounding astrocytes clear the glutamate by Na^+-coupled glutamate transporters, thereby elevating $[Na^+]_i$, and initially remove excess K^+ by mechanisms predominantly involving the combined action of the Na^+/K^+-ATPase isoforms. The Na^+/K^+-ATPase consists of an α- and a β-subunit, with several isoforms of each subunit present in the mammalian brain in unknown quantities and isoform combinations. As the isoform combination controls the functional characteristics of the Na^+/K^+-ATPase, we aim to determine their cellular distribution, favored combination, and abundance in rat astrocytes. Although the astrocytic α2β2 isoform constellation responds directly to $[K^+]_o$ above basal levels, it has been suggested that the astrocytic $[Na^+]_i$ may, rather, govern Na^+/K^+-ATPase activity and consequently control its ability to clear K^+ from the extracellular space. It is presently unknown to what extent astrocytic Na^+/K^+-ATPase activity is determined by $[Na^+]_i$ as the intracellular Na^+ affinity of isoform constellations involving the astrocytic β2 has remained elusive due to β1 presence in most expression systems and the technical challenges in measuring intracellular affinities in an intact system. We therefore expressed the different astrocytic isoform constellations in *Xenopus laevis* oocytes (α1β1, α2β1, α1β2, α2β2) and determined the apparent Na^+ affinity in isolated membranes as well as intact cells gradually loaded with Na^+. We subsequently defined the effect of the glutamate transporter-dependent increase in $[Na^+]_i$ on Na^+/K^+-ATPase-facilitated K^+ uptake, in primary astrocyte cell cultures. The obtained Na^+ affinities indicated that the Na^+/K^+-ATPase was near saturation at basal astrocytic $[Na^+]_i$, irrespective of isoform constellation, and was not stimulated by parallel glutamate transporter activity. Ongoing extracellular hippocampal slice recordings of stimulus-induced $[K^+]$ transients, using ion-sensitive microelectrodes, will reveal whether a glutamate

transporter-induced increase in $[Na^+]_i$ contributes to Na^+/K^+-ATPase-mediated K^+ clearance in a setting approximating native conditions.

T08-18A

Axo-glia interaction preceding CNS myelination is regulated by bidirectional Eph-ephrin signaling

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In the central nervous system, myelination of axons is required to ensure fast saltatory conduction and for survival of neurons. However, not all axons are myelinated, and the molecular mechanisms involved in guiding the oligodendrocyte processes towards the axons to be myelinated are not well understood. Only a few negative or positive guidance clues that are involved in regulating axo-glia interaction prior to myelination have been identified. One example is laminin, known to be required for early axo-glia interaction, which functions through α6β1 integrin. Here we identify the Eph-ephrin family of guidance receptors as novel regulators of the initial axo-glia interaction, preceding myelination. We demonstrate that so-called forward and reverse signaling, mediated by members of both Eph and ephrin subfamilies, has distinct and opposing effects on processes extension and myelin sheet formation. EphA forward signaling inhibits oligodendrocyte process extension and myelin sheet formation, and blocking of bidirectional signaling through this receptor enhances myelination. Similarly, EphB forward signaling also reduces myelin membrane formation, but in contrast to EphA forward signaling, this occurs in an integrin-dependent manner, which can be reversed by overexpression of a constitutive active β1-integrin. Furthermore, ephrin-B reverse signaling induced by EphA4 or EphB1 enhances myelin sheet formation. Combined, this suggests that the Eph-ephrin receptors are important mediators of bidirectional signaling between axons and oligodendrocytes. It further implies that balancing Eph-ephrin forward and reverse signaling is important in the selection process of axons to be myelinated.

T08-19A

Extracellular Vesicles (EVs) from leech microglia: a tool for understanding the dialog with damaged neurons

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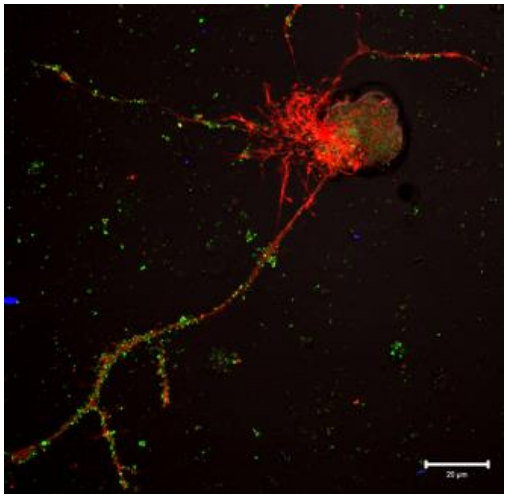
Because synapse regeneration spontaneously occurs in the leech nerve cord following microglial activation and accumulation, we hypothesize the existence of a particular neuroprotective microglial phenotype gathering at the lesion. Resident microglia are essential to the nerve repair since we know that they accumulate without any infiltrated blood cell to the lesion. Of interest, regeneration is mostly mediated by microglial cells because there are neither astrocyte nor oligodendrocyte in leech CNS.

Results obtained in microglia-neuron co-culture showed large amounts of Extracellular Vesicles (EVs)-like structures. In contrast, neurons cultured alone showed a significantly lower amount of EVs. Electron microscopy analyses confirmed the nature of ectosomes (100nm-1µm in diameter) and/or exosomes (50-100nm in diameter) of these structures. Of interest, time-course analyses of neuron morphology in purified neuronal cultures compared to neurons co-cultured with microglia indicated that microglia facilitate neurite outgrowth. The microglial origin of EVs was confirmed by using gliarin, a microglial marker known in the leech CNS. Indeed ten days culture of microglia with neurons showed the aggregation of microglial EVs immunopositive for leech gliarin (green) along growing neurites stained with phalloidin (red) (Figure). *Ex vivo* experiments from injured CNS showed the presence of EV accumulation at the lesion indicating that microglia would crosstalk with neurons through the release of EVs. The use of known mammalian vesicular markers also showed the presence of several

immunopositive EVs *i.e.* for Alix, C1q or CD11b. Finally, this neuroprotective microglial phenotype through EV production has been studied *in vitro* (neuron outgrowth assays) for a potential therapeutic approach in the regulation of nerve repair. The results showed that the leech microglia secretome containing EVs allows a robust sprouting of rat dorsal root ganglion (DRG) explant containing neurons (E18) compared to human NGF-treated DRG. The characterization of such EVs and their contents will lead to translational studies for the understanding of microglial neuroprotective roles.

Image



T08-20A

Astrocytic lactate release mediated by NH_4^+-dependent mitochondrial pyruvate shunting

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Ammonium (NH_4^+) has been present since the early history of the earth. This molecule had a key role in the origin of life, supporting the delivery of nitrogen for the development of unicellular organisms, plants and animals. However, for mammals is considered mostly a metabolic waste. Pathological levels of NH_4^+ are known to have profound effects on brain metabolism. Considering that in brain tissue NH_4^+ is produced by neurons in activity-dependent manner, the present work explored the possibility that NH_4^+ is a physiological modulator of astrocytic metabolism.

We have exploited the high spatiotemporal resolution of recently available FRET metabolite nanosensors to estimate intracellular lactate, glucose, pyruvate, NADH and ATP in mouse astrocytes *in vitro* and *in viv*o. Exposed to NH_4^+ levels known to be present in brain interstice, astrocytes in mixed cortical cultures and in acute hippocampal slices responded within seconds with a rise in cytosolic lactate, and its release to the extracellular space, as detected by a lactate-sniffer HEK293 cell. The astrocytic increase of lactate was also observed in the somatosensory cortex *in vivo*, in response to an intravenous infusion of NH_4^+. The lactate surge was not explained by glycolytic stimulation but by pyruvate shunting from mitochondria to lactate production caused by acidification of the mitochondrial matrix.

These results indicate that NH_4^+ redirects the flux of pyruvate from mitochondria to the production and release of lactate, and raise the possibility that NH_4^+ is a signal for neurometabolic coupling.

T08-21A

Cell-type specific responses to antidepressants - the epigenetic makeup of the glia-neuron interface

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Understanding the neurobiological underpinnings of major depressive disorder (MDD) is of utmost importance. A well-established tool to achieve this is the investigation of molecular responses to antidepressants (ADs). However, recently, ADs have been shown to act on neurons and astrocytes, calling for cell-type-specific approaches. Moreover, increasing evidence from human and animal studies suggest a pivotal role of astrocytes in restructuring neuronal networks, thus suggesting their potential relevance to reshape neuronal circuits that became miswired in depression.

Interestingly post-translational histone modifications, such as histone H3-lysine 4 trimethylation (H3K4me3) have been implicated as molecular mediators of AD efficacy, and show cell-type specific distribution patterns in healthy and diseased post mortem brain tissue.

In the present study we investigate the expression patterns of H3K4me3 in astrocytes and, for better comparison, in neurons, before and after treatment with fluoxetine (FLX), one of the most commonly used antidepressants. Furthermore, we included high anxiety-related behaviour (HAB) rats, which serve as a well validated animal model for depression, in our analysis. We are focussing on the prefrontal cortex (PFC), hence this brain region is known to be highly involved in depression. On that account, we use both *in vitro* and *in vivo* models. We demonstrate that under baseline conditions, untreated primary HAB astrocytes show 2-3 fold higher expression of H3K4me3 in comparison to wild-type (wt) astrocytes, by means of Western blotting experiments. In line with these findings, we observed increased H3K4me3 levels in the PFC of HAB animals in neurons as well as in astrocytes. After acute FLX treatment we detected an increase of H3K4me3 in the PFC of wt rats in neurons and astrocytes, while the methylation mark was decreased in HAB neurons.

Our results suggest that differences in H3K4me3 levels might occur in MDD like in HAB rats and that ADs act on astrocytes and neurons in a genotype specific manner. These changes might in turn induce plastic changes that correlate with therapeutic efficacy or individual AD resistance.

Currently, we are examining how the differing levels of H3K4me3 in wt versus HAB astrocytes might affect expression levels of genes, which are important for well-regulated neuronal function, and which could have potential protective value for the development of depression. Targeting those genes directly might be a valuable road to overcome AD resistance and side effects.

T08-22A

Circuit-specific signaling in astrocyte-neuron networks in basal ganglia pathways

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Astrocytes are non-neuronal cells that are emerging as important regulatory elements in brain function by actively exchanging signals with neurons. They respond to neurotransmitters and release gliotransmitters that modulate synaptic transmission. However, the cell- and synapse-specificity of the functional relationship between astrocytes and neurons in particular brain circuits remains unknown. Here we show that in the dorsal striatum, which mainly comprises two subtypes of intermingled neurons (striatonigral and striatopallidal medium spiny neurons, MSNs) and synapses belonging two

distinct neural circuits (the basal ganglia direct and indirect pathways), subpopulations of striatal astrocytes selectively respond to the activity of specific MSN subtypes. In turn, these subpopulations of astrocytes release glutamate that selectively activates NMDA receptors in homotypic, but not heterotypic MSNs. Likewise, subpopulations of astrocytes lead to the selective regulation of homotypic synapses through activation of group I metabotropic glutamate receptors. Therefore, bidirectional astrocyte-neuron signaling selectively occurs between specific subpopulations of astrocytes, neurons and synapses, which establish circuit-specific functional astrocyte-neuronal networks.

T08-23A

Changes of microRNA expression in glial cells of the amblyopic visual cortex submitted to modified geomagnetic fields

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The demonstrated participation of the small non-coding RNAs of interference (miRNAs) in nervous pathology prompted us to study changes of expression of these molecules in the visual cortex of dark-reared rats and submitted to modified geomagnetic fields. A microarray study of glial-specific miRNAs in this amblyopia model revealed changes of miR-34b and miR-338* expression. miR-34b is expressed by astrocytes, belongs to the mir-34/449 family and is a member of a genomic cluster together with miR-34c. In contrast, miR-338* is expressed by oligodendrocytes, belongs to the mir-338 family and is a member of a genomic cluster together with miR-3065. Lower expression of miRNAs is subsequently accompanied by an increase of their corresponding targets at the level of mRNA and protein. The significantly diminished expression of **miR-34b** in dark-reared animals is further increased after application of geomagnetic fields in which the horizontal component is rotated 120°. These changes implicate an increase of VAMP-3 (a) and Syt1 (b), both targets of miR-34b, and components of the neurosecretory vesicular molecules. **miR-338*** showed a significant decrease under the effect of modified geomagnetic fields. Proteic targets of miR-338* are NTF-3 (c) and CD-36 (d). Resulting increase of these proteins induces trophic effects and β-amyloid digestion, respectively. Based on these results, an alternative therapeutic approach that falicitates the entry of anti-miRNAs specific for miR-34b and miR-338* through the blood brain barrier into the brain could be designed (e).

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T08-24A

Microglia contribute to dendritic spine formation in postnatal mice somatosensory cortex

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Microglia are the immune cells of the central nervous system. Because of their immune properties, microglia have been focused on its functions onto injured or pathological condition. Over the last

decade, it has been revealed that microglia regulate synaptic function and number in physiological brain. Microglia survey brain parenchyma by extending and retracting their processes, selectively contact onto synapses and are also involved in selecting functional synapse through synapse elimination during injury and development in visual cortex, hippocampus and lateral geniculate nucleus. Thus, microglia may contribute in the functional neural circuit formation. In addition, microglia also involve in synapse formation during motor learning in adult mice motor cortex. In the developmental phase, microglia have activated phenotype. Then, activated microglia are known to release some molecules which related to synapse formation (e.g. brain derived neurotrophic factor, insulin-like growth factor). Additionally, activated microglia increase spine like protrusions at primary culture of developing preoptic area neurons. These all suggested the possibility of microglia to contribute on neural circuit formation during development. However, it is not clear whether microglia involved in synapse formation during development *in vivo*. To reveal this, we performed *in vivo* two photon imaging on the late postnatal mice which expressed EGFP in microglia and tdTomato in layer 2/3 pyramidal cells. We found that filopodia was formed at microglia contacted dendritic region during postnatal day 8-10 but not after postnatal day 12. We observed that actin accumulation for filopodia formation induced by microglia contact in slice culture. Intraperitoneal injection of minocycline, which inhibit microglia activation, reduced filopodia formation induced by microglia contacts *in vivo*. Spine density of minocycline injected or microglia ablated mice were significantly decreased, and miniature EPSCs frequency was also significantly reduced in microglia ablated mice. Taken together, these findings suggest that microglia contributed to synapse formation at somatosensory cortex during development.

T08-25A

Astrocyte molecular and functional heterogeneity in neural circuit formation

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Astrocytes are implicated in a growing number of neurodevelopmental diseases and are increasingly recognized as active participants in neural circuit formation. However, a limited appreciation of astrocyte heterogeneity has hampered efforts to understand their role during brain development. We have shown that astrocyte location within the central nervous system is one determinant of astrocyte heterogeneity. To identify novel astrocyte-encoded genes and functions, we used the transgenic mouse line Aldh1l1-eGFP to isolate early postnatal astrocytes by flow cytometry. We then performed gene expression profiling by microarray analysis of dorsal and ventral spinal cord and RNA sequencing of multiple brain regions (thalamus, hippocampus, subventricular zone, striatum, and cortex) to identify regionally heterogeneous astrocyte genes. One of these genes, the guidance cue Sema3a, is uniquely expressed by ventral spinal cord astrocytes and is required for proper motor neuron positioning and survival into adulthood. It also promotes normal synapse formation, and in its absence, motor neurons become hyperexcitable. Mice lacking astrocyte-encoded Sema3a had behavioral outcomes consistent with motor neuron depletion and hyperexcitability, respectively, suggesting that astrocyte-encoded Sema3a has multiple roles in promoting normal sensorimotor circuit formation. In ongoing work, we have identified a highly region specific gene signature in developing thalamic astrocytes, including candidate genes with potential roles in synapse development and pruning. In summary, expression profiling of regionally heterogeneous astrocytes is a powerfui tool for identifying novel astrocyte functions during mammalian brain development.

T08-26A

Energy metabolism of microglial cells

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The brain is a high energy-consuming organ. Multiple energy compounds in the cerebrospinal fluid (CSF) can serve as metabolic fuels for the intact brain, however, the exact compartmentation to different brain regions and cell types is still remain unclear. Under pathological conditions microglial cells can enter into metabolically different compartments in the CNS. We investigated, which of the compounds available in the extracellular space can be utilized by these cells.

The oxygen consumption rate (OCR) and extracellular acidification rate (ECAR) was measured on primary microglia and on the BV-2 microglial cell-line with Seahorse Extracellular Flux Analyzer (Seahorse). ECAR was considered as a parameter of glycolytic activity. Cells were incubated in Artificial Cerebrospinal Fluid (ACSF) supplemented with those substrates, available for the cells in the CSF: glutamine, glucose, lactate, keton bodies or pyruvate. ATP and ADP levels were measured using luciferin/luciferase bioluminescent method. Viability was detected with annexin/calcein fluorescent staining and MTT spectrophotometric assay.

All of the substrates applied supported the metabolism of the cells and none of them influenced their viability negatively. In the presence of glutamine and pyruvate the basal rate of respiration was increased and glutamine also increased the reserve respiratory capacity of the cells. However in the presence of glucose the OCR was decreased, the ECAR raised, indicating that glucose added to microglial cells stimulated glycolysis but inhibited oxidative metabolism. The addition of a lactate dehydrogenase inhibitor after glucose was able to reverse this effect. In the presence of glucose adding a mitochondrial fatty acid transporter inhibitor further increased the ECAR.

We conclude that microglial cells show high metabolic plasticity, using wide range of substrates. From the ECAR results we claim, that these cells show high glycolytic capacity. Furthermore it was found that besides glucose glutamine was the most preferred substrate for microglial cells.

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T08-27A

Radachlorin as a glia-specific photosensitizer

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Radachlorin, a chlorine-derived photosensitizer, is used in photodynamic therapy (PDT) of diverse tumors. We studied Radachlorin-PDT effect on peripheral neuron and glial cells. The isolated crayfish stretch receptor that consists of a single sensory neuron surrounded by glial cells a served as a simple model neuroglial preparation. Radachlorin accumulation and intracellular localization were studied using the fluorescence microscope. Neuronal activity was registered using standard electrophysiological methods. Necrotic and apoptotic cells were visualized using propidium iodide and Hoechst 33342. In the isolated crayfish stretch receptor Radachlorin localized predominantly to the glial envelope and penetrated very slightly into the neuron body and axon. Radachlorin accumulated rapidly in the crayfish nerve tissue within 30 min. Its elimination in the dye-free solution occurred slower: 11% loss for 2h. Radachlorin demonstrated very high photodynamic efficacy. Radachlorin-PDT inactivated the neuron and induced necrosis of neurons and glial cells and apoptosis of glial cells at as low as subnanomolar concentrations (10-11-10-9 M). It is, therefore, a promising glia-specific photosensitizer that kills glial cells through both necrosis and apoptosis. The study was supported by Russian Foundation for Basic Research (grants 14-04-32270 and 14-04-00741), and Russian Ministry of Education and Science (Research organization #790).

T08-28A

Glioendocrine system of thyroid hormone and its effect on microglia

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L-tri-iodothyronine (3, 3', 5-triiodothyronine; T3) is an active form of the thyroid hormone (TH) essential for the development and function of the central nervous system (CNS). We have reported non-genomic effects of T3 on microglial functions and its signaling (Mori et al., GLIA accepted). Exposure to T3 increased migration, membrane ruffling and phagocytosis of primary cultured mouse microglia. Injection of T3 together with stab wound attracted more microglia to the lesion site *in vivo.* The T3-induced microglial migration or membrane ruffling was dependent on TH transporters and receptors (TRs), $G_{i/o}$-protein, NO synthase, and subsequent signaling such as phosphoinositide 3-kinase (PI3K), mitogen-activated protein kinase (MAPK)/extracellular signal-regulated kinase (ERK). T3-induced microglial migration was also dependent on Na^+/K^+-ATPase, reverse mode of Na^+/Ca^{2+} exchanger (NCX), and small-conductance Ca^{2+}-dependent K^+ (SK) channel. Furthermore, we found that T3-indueced activation of glial cells in cortex and hippocampus were dependent on sex and age the animals. These results may help to understand physiological and/or pathophysiological functions of T3 in the CNS.

T08-29A

Effect of human pluripotent stem cell -derived astrocytes in the development and functionality of neuronal networks

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Question: Human pluripotent stem cells (hPSCs) can be differentiated into neural cell types and they are able to form functionally active networks. Growing evidence indicates that astrocytes can affect the neurodevelopmental process and maturation of hPSC derived neuronal networks. Currently, the effect of astrocytes on neuronal network formation and functionality is mainly studied using rodent models. However, human astrocytes have demonstrated critical differences in both structural and functional characteristics compared to rodent counterparts. The aim of the study is to address the significance of astrocytes to the formation, functionality and maturation of neuronal networks *in vitro* using human pluripotent stem cell derived cultures.

Methods: hPSCs were differentiated in neurosphere culture resulting to mixed population of neurons and astrocytes, where the switch from neuron enriched to astrocyte enriched culture takes place after extended culture time. The development of neuronal networks containing different proportion of astrocytes was characterized using fluorescent Ca^{2+} imaging and immunocytochemical staining.

Results: Functionally active networks can be formed with neuron enriched and astrocyte enriched cultures derived from hPSCs. Employing different research approaches: fluorescent Ca^{2+} imaging and immunocytochemistry we demonstrated functional maturation of the networks and individual neurons. Both neurons and astrocytes in networks show spontaneous and stimulated calcium signaling. Pharmacological characterization revealed differences in the functional properties of neuronal cells when cultured in mixed culture containing astrocytes.

Conclusions: Results suggest that astrocytes do have an affect both to development and maturation of neuronal networks and therefore should be considered as major contributor when developing *in vitro* platforms to study basic developmental processes as well as disease modeling.

T08-30A

Enhanced astroglial GABA uptake attenuates tonic $GABA_A$ inhibition of pre-sympathetic hypothalamic paraventricular nucleus neurons in heart failure

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γ-aminobutyric acid (GABA) generates persistent tonic inhibitory currents (I_{tonic}) and conventional inhibitory postsynaptic currents in the hypothalamic paraventricular nucleus (PVN) via activation of $GABA_A$ receptors ($GABA_A$Rs). We investigated the pathophysiological significance of astroglial GABA

uptake in the regulation of I_{tonic} in the PVN neurons projecting to the rostral ventrolateral medulla (PVN-RVLM). The I_{tonic} of PVN-RVLM neurons were significantly reduced in heart failure (HF) compared with sham-operated (SHAM) rats. Reduced I_{tonic} sensitivity to benzodiazepines and 4,5,6,7-tetrahydroisoxazolo[5,4-c]pyridin-3-ol in HF compared with SHAM neurons further confirmed HF I_{tonic} attenuation. HF I_{tonic} attenuation was reversed by a nonselective GABA transporter (GAT) blocker (nipecotic acid, NPA) and a GAT-3 selective blocker (SNAP-5114), but not by a GAT-1 blocker (NO-711). NPA and SNAP-5114 enhanced the I_{tonic} in HF compared with SHAM neurons, suggesting that GABA clearance increased in HF rats. Similar and minimal I_{tonic} responses to bestrophin-1 blockade using 5-nitro-2(3-phenylpropylamino)-benzoic acid (NPPB) in SHAM and HF neurons further argued against a role for astroglial GABA release in HF I_{tonic} attenuation. NPA inhibition of PVN-RVLM neuronal input resistance and input-output function was greater in HF than in SHAM rats, whereas bicuculine blockade of the I_{tonic} did not affect these parameters in HF neurons. As a result, the NPA-induced inhibition of spontaneous firing was greater in HF than in SHAM PVN-RVLM neurons. Overall, our results showed that the enhanced astroglial GABA uptake-induced attenuation of I_{tonic} in HF PVN-RVLM neurons explains the deficit in tonic GABAergic inhibition and increased sympathetic outflow from the PVN during heart failure.

T08-31A

Role of astrocytes in functional maturation of human neural network

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Question: Recent studies have indicated, that besides providing metabolic support for neurons, astrocytes have also more active roles in neural network function, such as participation in dendrite and axon outgrowth, formation and pruning of synapses, and removal of neurotransmitters from the synaptic cleft. Astrocytes have even been shown to be involved in synaptic transmission and plasticity. Furthermore, astrocytes are being recognized as contributors to several neurodevelopmental diseases, such as epilepsy and schizophrenia. Therefore, we hypothesize that astrocytes promote functional maturation of human pluripotent stem cell derived neural networks.

Methods: We applied our group's standard neural differentiation protocol on human pluripotent stem cells for 8 and 15 weeks, which produced neuron and astrocyte enriched cultures, respectively. The cultures were compared for gene (qRT-PCR) and protein (immunocytochemistry) expression. The main focus here is in microelectrode array (MEA) measurements, which records electrical activity in a neuronal network. The activity was analyzed for individual spikes and bursts of spikes with custom made Matlab algorithm. The networks were also analyzed with Ca2+-imaging.

Results: The neural enrichment produced a network with 60% of neurons and 10% of astrocytes, while the remaining portion of cells remained mainly as neural precursors. The astrocytic enrichment yielded 55% of neurons and 35% of astrocytes. The detailed analysis of vast MEA data sets is currently ongoing, but initial analysis of spike counts suggest that in the astrocyte enriched culture there are less electrically active areas. However, the active areas in the astrocyte enriched culture showed more activity than in the neuron enriched culture.

Conclusions: The results suggest that astrocytes prune weak connections from the neural network while enhancing strong ones. Accordingly, astrocytes should be taken into account when developing in vitro disease models as they are part of normal neuronal network development.

T08-32A

Modeling neuron-astrocyte interactions at network level

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There have been many studies and models concentrating on tripartite synapses formed by two neurons and an astrocyte. The effects of tripartite synapse on paired pulse facilitation and depression

were suggested for example by De Pittá et al. (PLoS Comput. Biol. 2011). This signaling pathway consists of glutaminergic synapses where the astrocyte detects the glutamate and responds with intracellular IP3 and calcium signals. In response to intracellular calcium changes the astrocytes release gliotransmitters that are detected by extrasynaptic receptors which leads to a kind of feedback loop. In another study by Lallouete et al. (Front. Comput. Neurosci. 2014.) the spreading of intracellular calcium waves was studied in silico. Their results show that the topology astrocyte network is important for calcium wave spread distance. We aim to get one step further and study the astrocyte effects on neuronal network level.

Methods: We use phenomenological neuronal network simulator called INEX by Lenk (2011). Each neuronal connection has a synaptic strength and each neuron has a spiking probability based on basic activity of the neuron and the sum of inputs it gets from other spiking neurons. In order to study the effects of astrocytes to a neuronal network we introduce a scheme where the model is combined with the Tsodyks-Markram synapse simulator used also by De Pittá et al. and astrocyte network calcium spreading UAR model by Lallouette et al. The simulators are combined in a biologically reasonable way in order study possible astrocyte signaling effects to neuronal network behavior. The strength of the neuron-astrocyte-neuron connections are modelled at multiple levels starting with local calcium dynamics parameters and local calcium effect contribution to whole cell signaling, to gliotransmissions and their effects. Combining the models produce an interconnected network of neurons and astrocytes having different spatial and temporal dynamics. Here we study the short term dynamics and look both neuronal spiking dynamics and astrocyte Ca activity.

Results: The results show that amounts of astrocytes as well as strength of neuron-astrocyte-neuron signaling have profound and nonlinear effects to network spiking behavior. Astrocyte strength and amount of astrocytes are not interchangeable variables but have different effects. Thus increasing number of astrocytes to have higher interconnectivity between the two networks does not have the same effect as having less but stronger astrocytes.

T08-33A

Microglial CX3CR1 deficiency delays the maturation of adult born neurons in the olfactory bulb

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Background: Over the past decade it became evident that microglia play important roles in brain development and plasticity. Specifically, previous studies showed that during brain development microglia actively participate in the proliferation and differentiation of newborn cells, as well as in synapse pruning, a crucial step in brain circuitry development. These developmental roles of microglia were found to be mediated by the fractalkine/CX3C receptor 1 (CX3CR1) signaling pathway. This pathway plays a major role in neuronal-microglial interactions, in which neuronally-derived fractalkine signals via CX3C receptors, which in the brain are exclusively expressed by microglia. Despite their importance in developmental processes, the role of microglia and CX3CR1 signaling in the maturation, dendritic and synapse growth of newly-born neurons during adult neurogenesis is still unknown. **Methods**: To elucidate the role of CX3CR1 signaling in neurogenesis we labeled newborn neurons migrating to the olfactory bulb (OB) in mice with CX3CR1 deficiency and their WT controls, using a lentivirus that induces the expression of tdTomato in infected host cells. 28 days after the injection we imaged infected maturing peri-glomerular neurons in the OB using 2-photon microscopy, followed by immunohistochemical examination of enhanced tdTomato labeling, measuring the spine density and size in maturing OB granular cells. **Results**: Histological data, obtained from n=20 cells from 5 mice in each group, shows that the spine density on granular cell dendrites in mice lacking microglial CX3CR1 was significantly lower as compared to WT controls. Moreover, the spine morphology differed between the groups, as the CX3CR1 deficient mice had significantly smaller spines. Preliminary findings form the in vivo analysis (obtained from n=8 cells from 2 mice in each group) show that peri-glomerular neurons in CX3CR1-deficient mice had longer dendrites as compared to WT controls. **Conclusions**: Our findings suggest that microglia are involved in normal maturation of adult newborn neurons in the OB and that fractalkine/CX3CR1 signaling deficiency causes a alteration and/or delay in the wiring of newborn cells into the network.

Image

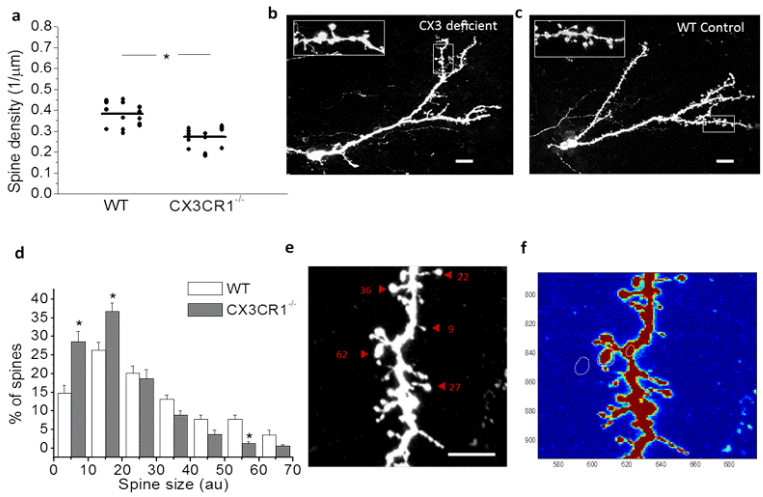

Fig.1. **Reduced spine density and synaptic maturity in 28 day old adult-born granule cells of CX3CR1 deficient mice.**
a. Adult born granule cells(GCs) of CX3CR1 deficient (CX3CR1 -/-) mice have less spines than of WT controls. * p< 0.001. n=20 GCs; n=5 mice in each group. **Images b-c**. Confocal micrographs (projection image) of adolescent adult-born granule cells expressing tdTomato, 28days of age. Scale bar: 20 µm. . **d.** Spine size distribution of adult born granule cells in CX3CR1 deficient (CX3CR1 -/-) mice is skewed towards smaller spines. * p<0.01,ttest with corrected alpha. n= 801 spines from 13 GCs of WTs and n= 781 spines from 14 GCs of CX3CR-/- . N=4 mice. All values are mean±SEM. . **Images e-f.** (Left) High resolution confocal projection of one dendritic segment analyzed for spine head sizes. Four representative spine heads are marked with red arrow heads and their size values by numbers . Larger numbers indicate larger spine heads. (Right) An example analysis of one spine (color intensity image). The regions marked for analysis are : head, adjacent background and adjacent dendritic shaft. Scale bar: 10 µm.

T08-34A

Glial cells influence synaptic plasticity of competing nerve terminals and alter the outcome of synaptic competition at the mammalian neuromuscular junction

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Question: The precise wiring of synaptic connections is shaped by elimination of supernumerary inputs competing for the innervation of the same target cell. At the neuromuscular junction (NMJ), this competition depends on the synaptic efficacy of competing terminals which strengthens one input and weakens the others, leading to their elimination. However, little is known about the synaptic activity and plasticity of competing terminals. Moreover, the role of glial cells during synaptic competition remains ill-defined despite their importance in the modulation of synaptic efficacy and plasticity at adult NMJs. Therefore, the goal of this work was to study synaptic plasticity of strong and weak terminals during synaptic competition and their interaction with perisynaptic Schwann cells (PSCs), glial cells at NMJs.

Methods & results: We performed intracellular recordings from dually innervated P7-8 mouse Soleus muscle fibres to assess synaptic activity and monitored PSC activity using confocal Ca^{2+} imaging. PSCs were loaded using single cell electroporation of the Ca^{2+} indicator Fluo-4 and the morphological dye Alexa 594. PSCs decode synaptic competition as revealed by tight relationship between the size of Ca^{2+} responses and the synaptic strength of each input (i.e. weak input generated smaller Ca^{2+} responses than the strong one). At the same NMJ, the strong input showed a long-lasting potentiation of neurotransmission while the weak one displayed only a small transient potentiation. To determine whether the differential plasticity of competing terminals was related to PSCs Ca^{2+} responses, single PSCs were blocked by photoactivation at 405 nm light of Diazo-2 (photoactivable BAPTA) electroporated in PSCs. Photoactivation of Diazo-2 blocked PSCs Ca^{2+}-responses and prevented synaptic plasticity. However, direct induction of large, but not small Ca^{2+} responses (mimicking activation by strong inputs) with the Ca^{2+}caged compound NP-EGTA resulted in a long-lasting potentiation of the strong input while the weak terminal displayed only a transient one. Finally, the

period of synaptic competition was extended when the ability of PSCs to decode synaptic competition was blocked in vivo using the P2Y1 receptor antagonist MRS2179.

Conclusion: These data indicate that glial cells decode synaptic competition at NMJs and govern synaptic plasticity of competing nerve terminals. The differential plasticity of strong and weak inputs influences the outcome of synaptic competition and elimination.

T08-35A

Imaging dynamics of energy metabolites in hippocampal astrocytes during neuronal activity

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Little is known about the dynamics of energy metabolites during neuronal activity, partly because the current isotopic techniques do not have enough spatio-temporal resolution to discriminate cell types. Attributable to the development of new genetically-encoded optical FRET nanosensors for glucose (*Takanaga et al., 2008*) and lactate (*San Martin et al., 2013*), it is now possible to measure the dynamics of these energetic metabolites in single cells with a resolution of seconds.

The aim of this work was to evaluate the impact of neuronal activity on the intracellular glucose and lactate in astrocytes of hippocampal mouse organotypic slices, transduced with adenoviral vectors containing the sensors for glucose or lactate. Focal electrical stimulation (20 Hz) of Schaffer collateral fibers caused a fast, reversible and TTX-sensitive fall of glucose and lactate in astrocytes. Recurrent network activity induced in a Mg^{2+}-free, bicuculline-containing saline resulted in similar metabolite changes in astrocytes. To explore the neuronal signal(s) responsible for the astrocytic activation, we superfused slices with glutamate or K^+. Glutamate (100 µM) did not change the rate of glycolysis within seconds, but caused a reversible increase in intracellular lactate. Increasing the extracellular K^+ concentration (12 mM) caused glycolytic activation and lead to a fast and reversible drop in intracellular lactate, which was insensitive to the specific MCT blocker AR-C 155858.

Taken together, these data suggest that astrocyte metabolism is highly sensitive to neuronal activity, and K^+ seems to be the neuronal signal which initiates the fast glycolytic activation and lactate depletion. This findings support recent evidence for an astrocytic ion channel which rapidly mobilizes lactate upon membrane depolarization (*Sotelo et al 2015*)

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T08-36A

Analysis of astrocyte-specific and inducible $GABA_B$ receptor deletion in the mouse brain

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Astrocytes are important regulators of synaptic transmission. For this purpose, they are equipped with a plethora of transmitter receptors that sense neuronal activity.

Here, we are investigating the role of the metabotropic $GABA_B$ receptor, a sensor of the main inhibitory neurotransmitter g-aminobutyric acid (GABA), in conditional knockout mice (cKO) generated by crossbreeding GLAST-CreERT2 x $GABABR1^{fl/fl}$ mice.

Genomic DNA recombination, i.e. gene deletion, was induced by intraperitoneal tamoxifen injection and quantified by quantitative real-time PCR after three weeks. Analysis of genomic DNA purified from cerebellum, cortex and hippocampus revealed a reduction of floxed *gabbr1* alleles (14 %, 25 % and 29 %, respectively) that reflected the relative proportion of astroglia in the respective CNS region. However, quantification of GABABR1 mRNA expression in cKO and control mice did not show significant differences, indicating the predominant expression of GABABR1 in neurons rather than astrocytes. However, using high-resolution confocal laser-scanning microscopy of immunostained brain sections, we could observe a significant reduction of GABABR1 protein from astrocyte membranes.

A subset of our GLAST-CreERT2 x $GABABR1^{fl/fl}$ mice also expresses the genetically encoded Ca^{2+} indicator GCaMP3. By *in vivo* 2-photon laser-scanning microscopy of awake mice we are now exploring the impact of astroglial $GABA_B$ receptors for neuron-glia communication and behaviour.

T08-37A

Localization of several acid-base regulating, lactate transporting proteins and Carbonic Anhydrase II in astrocytes & neurons in mouse hippocampus

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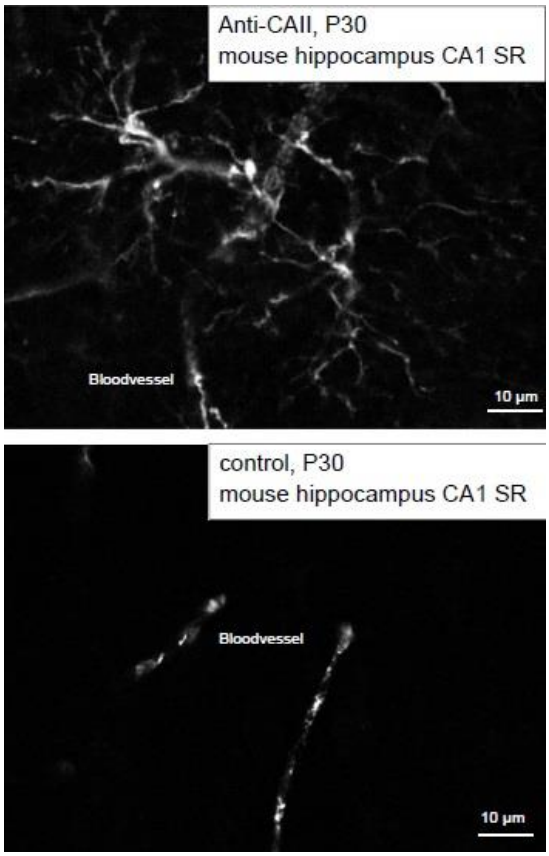
The knowledge about the interplay between astrocytes and neurons concerning energy support and pH-regulation in an adult and healthy brain has changed during the last decade. Energy- and pH-regulation are closely connected with each other because high metabolic activity during neuronal activity leads always to pH-changes - in neurons as well as in astrocytes. Carbonic anhydrase II is involved in these processes, forms a transport metabolon with some of the acid/base regulating proteins generally expressed in astrocyte membranes or interacts by being close enough. Main goal of this project was to show proper expression of the proteins of interest: Astrocytes are equipped to nourish neurons with lactate and regulating most of the acid/base disturbances upon neuronal activity. Neurons must be able to take up the energy molecules and have to possess proteins to regulate (intracellular) pH-changes.

Monocarboxylattransporter 1 (MCT1), involved in lactate transport, sodium-proton-exchanger 1 (NHE1), responsible mainly for regulation of acid loads and the mobile enzyme carbonic anhydrase II (CAII) were visualized in different cells of CA1-region of mouse hippocampus by using antibodies. Age dependent, 250µm thick acute slices of BL6-mice were compared in their protein expression patterns with a CAII-knock out strain, in order to elucidate differences in protein expression profile or cell shape. In this context neurons and their expression of MCT2 and NHE5, as well as CAII and NHE1 were compared, too. These factors might indicate cellular variation leading to altered astrocytic or neuronal functioning. Additional a density gradient centrifugation method for isolation of synaptic vesicles was used, in order to analyze the protein expression on level of synapses within the framework of the Western Blotting Method.

All slices, WT and KO, revealed a positive expression of NHE5 and MCT2 in neurons and NHE1 and MCT1 were detected in both, neurons and astrocytes. NHE1 showed, independent from genotype and age, the most prominent and constant expression. A co-localization of CAII with the NHE1 and the MCT1 was positively established in astrocytes. By supporting previous results, the blots showed MCT2-, NHE1- and NHE5-expression in synaptic end terminal, where PSD-95 was found either. Furthermore CAII was verified in the synaptosomal fraction. CAII seems to be a mobile enzyme, whose localization has to be revealed by further experiments, like subcellular imaging and electrophysiology, where a distinction between young and old animals is either necessary.

Image



T08-38A

A nonsense point mutation in a novel SLC25 family member of mitochondrial carriers causes severe recessive neurological disease and epilepsy in mice

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SLC25 is the largest Solute Carrier family of nuclear-encoded transporters embedded in the inner mitochondrial membrane, where they shuttle a variety of metabolites across it. The members of this superfamily are widespread in eukaryotes, and are involved in numerous metabolic pathways and cell functions, varying mainly in the nature and size of their transported substrates. Many members of the family are expressed in the CNS, and appear to play a key role in energy production and neurotransmission in healthy neurons. Mutations in SLC25 family genes impair normal mitochondrial function and have therefore been implicated in neurodegenerative diseases such as Parkinson, Alzheimer and ALS.

Following a forward genetics approach through random mutagenesis, we identified a novel mouse model of severe autosomal recessive neurological disease, characterized by ataxia, tonic-clonic seizures, unsteady locomotion, conscience loss and growth retardation with early lethality. Epileptic seizures occur frequently in mitochondrial diseases, linked to an increased number of nuclear gene defects. In line with the ataxic phenotype, morphological analysis revealed pronounced abnormalities in the cerebellum, including: a) a narrower molecular layer with impaired dendritic arborization of Purkinje cells (PCs); b) reduced vesicular distribution of parallel fiber-PC synapses and climbing fiber-PC synapses as testified by immunoreactivity of the vesicular glutamate transporters vGLUT1 and vGLUT2; c) a reduction in the PC calcium binding protein calbinding.

Based on the seizure phenotype here we examined the hippocampus, which is a major area of glial response involving astrocytes and microglia. In 4-week old mice we observed a) an increase in the number of $GFAP^+$ astrocytes and $IBA1^+$ microglia cells in the dentate gyrus of mutant mice; b) an increase in $Ki67^+$ proliferating cells and $Ki67^+/DCX^+$ cycling neuroblasts; c) a reduction in the number of DCX^+ (doublecortin) newly born neurons and d) a decrease in the number of PV^+ (parvalbumin) mature neurons. Our data indicate aberrant neurogenesis in the dentate gyrus of the hippocampus and possible astrocytic dysfunction both of which may contribute to the epileptic phenotype. Further studies of the neuroglial interactions in this system are under way.

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T08-39A

The role of glial lipid metabolism in synaptic plasticity

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The brain is considered to be autonomous in lipid synthesis because of the blood-brain barrier that prevents many types of lipids (e.g. cholesterol, phospholipids, saturated fatty acids) from entering the brain. *In vitro* studies have shown that astrocytes produce lipids more efficiently than neurons, and that astrocyte-derived lipids are taken up by neurons to support neurite outgrowth, synapse formation and function. We previously tested this concept *in vivo* by generation of mice with a deletion of the SREBP cleavage activated protein (SCAP), a gene required for cholesterol and fatty acid synthesis, in astrocytes and reported that these mice show reduced levels of cholesterol and specific fatty acids in the whole brain accompanied by brain hypotrophy and defects in motor control and behavior (Camargo et al., 2012). Here we assessed the role of astrocyte-derived lipids on hippocampal synapses. For this purpose, we determined hippocampal synapse number and maturation, ultrastructure and protein expression, as well as synaptic function and hippocampal-based learning of astrocyte SCAP mutant animals. This study will help to better understand the role of lipids in the brain and may be of great advantage in dietary treatment of cognitive deficits linked to reduced lipid synthesis.

T08-40A

BDNF effect on LTP is modulated by astrocytes in rat hippocampus

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Hippocampal θ-burst long-term potentiation (LTP) is a form of synaptic plasticity, which can be enhanced by BDNF (Fontinha et al, Neuropharmacology, 2008). It is well known the active role of astrocytes on synaptic plasticity, since Ca^{2+}-dependent gliotransmitters release from astrocytes play a crucial role in hippocampal LTP induction (Henneberger et al., Nature, 2010). The main described mechanisms for modulation of LTP by BDNF occur in neurons, being neglected the possible involvement of astrocytes for this phenomena. Thus the aim of the present work was to investigate the role of astrocytes upon the potentiation of hippocampal LTP magnitude by BDNF.

fEPSP were recorded from the CA1 area of hippocampal slices prepared from Wistar rats (3-5 weeks old). Two distinct magnitude-LTPs were induced by theta-burst protocol in the Schaffer collaterals/CA1, namely a 10 trains separated by 200 ms, four pulses each, 100Hz (strong LTP) and a 3 trains separated by 200 ms, three pulses each, 100Hz (mild LTP).

The strong and mild θ-burst stimulation increased the slope of fEPSP by 44±10% and 16±5.9% respectively, whereas in the same slices but in the presence of BDNF (20ng/ml) the same induction paradigm enhanced fEPSP slope by 87±15% and by 39±4.7%, respectively. For both θ-burst stimulation paradigms, LTP was completely abolished when hippocampal slices were superfused with the gliotoxin fluorocitrate (FC), which reduces the astrocytic metabolism. In presence of FC we observed an enhancement of LTP magnitude mediated by BDNF application only for strong LTP induction. When a mild LTP was induced, BDNF application was not able to enhance LTP.

Since activation of adenosine A2A receptor is crucial for BDNF mediated effects on LTP (Fontinha et al, 2008), we hypothesised that the adenosine involved in this processes was being provided by astrocytes. Thus we investigated if activation of adenosine A_{2A} receptor ($A_{2A}R$) could rescue BDNF effect on LTP under induced metabolic arrest of astrocytes by using the gliotoxin FC. When slices were superfused with the selective $A_{2A}R$ agonist, CGS21680 (30nM), and FC the mild θ-burst stimulation decreased the slope of the fEPSP by 18±2.8%, whereas in the same slices but in the presence of BDNF the same induction paradigm enhanced the fEPSP slope by 11±5.7% ($p<0.05$).

These results demonstrate that under mild θ-burst stimulation BDNF is able to induce LTP however the effect is controlled by adenosine/ATP provided by astrocytes. Under strong θ-burst stimulation the enhancement of LTP mediated by BDNF is not under control of astrocytes.

T08-41A

Revealing the locally translated mRNA repertoire at synapses between neurons and NG2-expressing glial cells

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Neuronal signal transduction occurs via synapses consisting of presynaptic and postsynaptic neuronal compartments. However recent studies have defined synapses formed between neurons and glial cells expressing the chondroitin sulphate proteoglycan NG2 (NG2+ cells, 1,2). NG2+ cells are migratory (3), and are the precursors of myelinating oligodendrocytes (4). In the developing and adult brain, they are found in both grey and white matter. The cells express ionotrophic AMPA and GABAAreceptors which can transmit synaptic input to the NG2 cells. With ensuing differentiation to Myelin Basic Protein-expressing myelinating oligodendrocytes, expression of NG2 is lost and concomitantly the cells lose synaptic contacts to neurons (5). It has been reported that NG2+ cells receive glutamatergic signals via calcium permeable channels in the hippocampus (6). The synaptic contact between a presynaptic axon and a postsynaptic NG2 cell was also demonstrated by electron microscopical analysis that shows NG2 processes in proximity to synaptic vesicle-containing presynaptic axons (7). It has been recently reported that glutamatergic synapses from thalamocortical neurons regulate the proliferation and mobilization of NG2+ cells (8) and the differentiation of NG2 cells to oligodendrocytes is enhanced by physical activity (9) .

Despite these studies, the physiological role of neuron-glia synapses remains to be elucidated. Our aim is to identify molecular components of NG2 cells as a receptive postsynaptic element by isolating such glial-neuronal compartments using biochemical fractionation and cell sorting. Locally translated mRNAs such as Myelin Basic Protein (10) are being characterized by taking advantage of high-throughput sequencing. The role of defined mRNAs will be investigated and changes in response to neuronal stimuli will be studied.

T08-42A

Disturbances in microglial functioning underlie stress-induced depressive-like behavior and suppressed neurogenesis

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Question: The limited success in understanding the pathophysiology of major depression may result from excessive focus on the dysfunctioning of neurons, as compared with other types of brain cells. **Methods**:Therefore, we examined the role of dynamic alterations in microglia activation status in the development of chronic unpredictable stress (CUS)-induced depressive-like condition in rodents. **Results**:We report that following an initial period (2-3 days) of stress-induced microglial proliferation and activation, some microglia underwent apoptosis, leading to reductions in their numbers within the hippocampus, but not other brain regions, following 5 weeks of CUS exposure. At that time, microglia displayed reduced expression of activation markers as well as dystrophic morphology. Blockade of the initial stress-induced microglial activation by minocycline or by transgenic interleukin-1 receptor antagonist over-expression rescued the subsequent microglial apoptosis and decline, as well as the CUS-induced depressive-like behavior and suppressed neurogenesis. Similarly, the antidepressant drug imipramine blocked the initial stress-induced microglial activation as well as the CUS-induced microglial decline and depressive-like behavior. Treatment of CUS-exposed mice with either endotoxin, macrophage colony stimulating factor (M-CSF) or granulocyte-macrophage colony stimulating factor (GM-CSF), which all stimulated hippocampal microglial proliferation, partially or completely reversed the depressive-like behavior and dramatically increased hippocampal neurogenesis, whereas treatment with imipramine or minocycline had minimal or no anti-depressive effects, respectively, in these mice. **Conclusions**:These findings provide direct causal evidence that disturbances in microglial functioning has an etiological role in chronic stress-induced depression, suggesting that microglia stimulators could serve as fast-acting anti-depressants in some forms of depressive and stress-related conditions.

T08-43A

Contactin-2/TAG-1 affects oligodendrocyte populations and CNS myelination

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The molecular architecture of the myelinated fiber and the specialized interactions between the axon and the myelinating glia are important for the homeostasis of the axon and efficient nerve conduction. Contactin-2/TAG-1 a member of the IgSF, is expressed by neurons during development, while in the adult CNS and PNS is expressed by axons and glial cells. There, it is localized at the juxtaparanodal area of the myelinated fiber where it interacts with Caspr2 and the VGKCs. Our previous studies showed that Contactin-2 is indispensable for the formation and the maintenance of the juxtaparanodal complex since its loss leads to Caspr2 and VGKC lack of clustering and diffusion towards the internode. In addition, Contactin-2 deficient animals exhibit hypomyelination of the optic nerve and behavioral deficits. Contactin-2 was recently identified as an auto-antigen in a subset of MS patients and it is believed to be implicated in gray matter pathology. The results from both rodent models and human postmortem tissue point out at the implication of Contactin-2 both in CNS myelination and pathology, although its precise involvement remains unknown. Here we are investigating the role of Contactin-2 in oligodendrocyte development and myelination under normal and pathological conditions. Taking advantage of a Contactin-2-GFP transgenic animal, we saw expression only by mature oligodendrocytes and not by oligodendrocyte progenitors (OPCs). We asked how the absence of Contactin-2 can affect oligodendroglial population numbers and morphology, myelin gene transcription and protein expression by comparing normal and Contactin-2 deficient animals during multiple time points of the myelination process. In parallel, using the cuprizone model of toxic CNS demyelination, we asked if the absence of Contactin-2 can differentially affect the oligodendroglial population, their remyelinating potential, conduction velocities and finally animal behavior when compared to the wild type/untreated controls. We found that Contactin-2 loss affects oligodendrocyte numbers, myelin protein levels and the transcriptional profile in the cortex Under demyelination conditions, the absence of Contactin-2 affects mature oligodendrocyte numbers and OPC recruitment to the lesion area; moreover, Contactin-2 deficient animals fail to remyelinate as efficiently as wild type littermates, underscoring the importance of Contactin-2 both in myelination and related pathology.

T08-44A

Astrocytic networks are determinant for generation of rhythmic bursting by assemblies of trigeminal neurons involved in mastication

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Rhythmic movements like locomotion, respiration and mastication are vital functions that are executed with a high level of "automaticity" by neuronal networks often called central pattern generators (CPGs) that can generate rhythmic motor activity patterns and constantly adjust those patterns in response to specific sensory stimuli. The mechanisms underlying both generation of rhythmic activity (rhythmogenesis) and adjustment of this activity in function of sensory inputs are still poorly defined in most CPGs. There is considerable evidence that the trigeminal main sensory nucleus (NVsnpr), which receives sensory inputs and projects to motoneurons and premotor interneurons, contains the neurons forming the core of the masticatory CPG. NVsnpr neurons fire rhythmically in response to stimulation of the sensory inputs to the nucleus or to local NMDA applications, and the ionic current responsible for their rhythmic firing (I_{NaP}) is modulated by the extracellular concentration of Ca2+ ([Ca2+]e). Our previous work has shown that rhythmogenesis in NVsnpr heavily depends on interactions between neurons and astrocytes because inactivation of the astrocytic network by intracellular dialysis with BAPTA prevents neuronal bursting, suggesting that integrity of the astrocytic network is important. Here we examine first, if stimuli that induce bursting in neurons have an effect on astrocytes and influence their coupling, and second if astrocytes coupling determines the firing pattern and the synchronization of NVsnpr neurons. We show that stimulation of afferents or NMDA local applications increase intracellular Ca2+ of astrocytes, depolarize them and increase coupling between them as evidenced in dye coupling experiments. The astrocytic networks revealed remain confined within the region of NVsnpr where neurons fire rhythmically and were mostly oriented towards the center of this region. NMDA-induced neuronal bursting was abolished by Carbenoxolone (20μM), a blocker of gap junctions. Once blocked, this bursting could be rescued by local application of an astrocytic Ca2+-binding protein. This work suggests that in addition to regulating the extracellular ions concentrations which determine the neuronal firing pattern of NVsnpr neurons, astrocytes may also play an important role in synchronizing entire assemblies of neurons in this nucleus.

T08-45A

Investigating glia-neuron cross-talk during Parkinson´s disease pathogenesis using patient-specific iPSC-derived cells

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Our understanding of Parkinson's disease (PD) pathogenesis, the second most common age-related progressive neurodegenerative disease, remains elusive despite decades of thorough investigation, arguably owing to the lack of suitable experimental models recapitulating the disease. Induced pluripotent stem cells (iPSC) derived from somatic cells of patients are an innovative tool for *in vitro* modeling of complex diseases and may also provide a source for cell replacement therapies. It has been recently demonstrated that iPSC technology can be used to observe disease-associated phenotypes relevant to PD neurodegeneration, in particular impaired axonal outgrowth and deficient autophagic vacuole clearance. iPSC disease modeling has provided first hand proof-of-principle evidence that neurons with a sporadic PD patient genome exhibited similar phenotypes compared to ones derived from patients with familial PD. Our group has successfully derived both patient-specific dopaminergic neurons (DAn) and glial cells from iPSC. In the present study we aim to delve into the investigation of glial cells to discern whether DAn degeneration in PD is truly a cell-autonomous phenomenon, or whether it is influenced by an altered cross-talk between DAn and glial cells. We are now investigating the effects of co-culturing different iPSC-derived patient-specific DAn/astrocyte combinations from PD and control patients. After glial characterization, PD specific associated phenotypes, such as alpha-synuclein accumulation and alterations in autophagic machinery, have been observed in our cells. By recapitulating the accurate neurodegenerative phenotypes seen in PD pathology in a progressive manner through the use of iPSC technology, several unknown mechanisms will be unveiled to aid in the future development of specific PD-targeted therapies.

T08-46A

Myelin and cognition: beyond conduction velocity

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The role that myelin has on cognitive processes is not well understood beyond its speeding up of action potential propagation. We tested the role of myelin on both auditory sensory processing and handedness in a mouse model (*shiverer*) with severe dysmyelination in the absence of axonal degeneration. Since the auditory system is highly optimized for the processing of stimuli that are encoded in time, we hypothesized that auditory processing would be strongly affected in the absence of myelin. Auditory processing was assessed with acute electrophysiology in the auditory system of *shiverer* and wild type (wt) mice, while presenting stimuli patterns aiming to test both frequency and temporal coding. In parallel, since the corpus callosum is a long and well myelinated cortical fiber path, we expected its functionality to be impaired in dysmyelinated mice. Corpus callosum function was assessed through handedness, using a well-established test for its integrity, the paw preference test.

Frequency discrimination was surprisingly unimpaired in the auditory system of *shiverer* mice, despite a clear and expected increase in response latency. Temporal processing, on the other hand, was clearly impaired. We used two standard protocols for temporal processing measurements: gap detection (acuity) and click rate following (reliability). *Shiverer* mice had higher gap detection thresholds and could not follow click rates as well as *wt.* This reductionin acuity and increased response fatigue was accompanied by abnormally large responses. These effects cannot be exclusively attributed to an increased response latency.

Lateralization was also very different between the wt and the shiverer mice. While *wt* mice were strongly lateralized and had a symmetrical distribution of paw preference, *shiverer* mice were significantly less lateralized and had a shift in handedness, with a tendency to be either ambidextrous or left handed. The data suggest that stable lateralization is dependent on the presence of myelin and opens questions about the mechanisms underlying efficient lateralization and interhemispheric interactions.

Shiverer mice, which have severe dysmyelination, show sensory processing deficits and handedness changes that are dificult to explain only by changes in conduction velocity. While myelin thickness is clearly a well regulated aspect of myelination, other aspects, such as its coverage (internodal length) and its spacing (nodal lengths) are likely to play important, but to date not well understood, roles in neuronal excitability and reliability.

T08-47A

Cerebral glucose uptake measurements on a single cell level reveal higher transport in astrocytes *in vivo*

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To cover its high energetic expenditures, the brain mainly utilizes blood-borne glucose. In the past, a vast array of methods such as the 2-deoxyglucose method or magnetic resonance spectroscopy-based approaches have been used in the living organism to estimate glucose metabolism-related processes. However, given that strict cellular compartmentation of brain energy metabolism has been theorized, methods enabling measurements of metabolic aspects with higher spatiotemporal resolution would be valuable.

Recent implementation of genetically encoded glucose sensors provides the opportunity to address metabolic compartmentation at the cellular level. By expressing the glucose sensor FLII^{12}Pglu600μΔ6 in cerebral cortex of C57BL/6 mice and using two-photon laser scanning microscopy, we were able to measure glucose concentrations in single astrocytes and neurons *in vivo*. After setting a low glucose

baseline level by using insulin, we followed glucose transport into individual brain cells and the extracellular space upon intravenous glucose administration.

We observed that glucose levels equilibrated faster between neurons and astrocytes than between blood and tissue suggesting that transendothelial passage is the main limiting barrier for glucose uptake. Despite the temporally homogenous behavior between neurons and astrocytes, the latter exhibited higher glucose transport upon glucose administration (23.6 ± 8.2 % vs. 11.8 ± 5.4 % signal change 30 minutes after glucose injection). In the last part of our work, we expanded the use of the technique to the awake head-fixed mouse. Interestingly, licking of glucose solution led to accumulation of glucose in astrocytes but no signal increase in neurons was observed.

In summary, the feasibility of this novel imaging approach offering single cell resolution in the intact animal is demonstrated. Our results support previous work suggesting a cellular compartmentation of cerebral glucose transport with higher glucose uptake in astrocytes.

T08-01B

GLAST-CreERT2/KOeif2b a relevant mouse model for the CACH/VWM leukodystrophy

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Glial cells have been identified as the primary target of vacuolating leukodystrophies (LKD) related to GFAP or MLC1 mutations. Mutations in the ubiquitous translation initiation factor eIF2B, which is involved in regulating global protein synthesis particularly in stress conditions, induced a recessive form of vacuolating LKD named vanishing white matter disorder (VWM) or childhood ataxia with central nervous system hypomyelination (CACH) based on cerebral MRI and clinical signs. A wide clinical spectrum ranging from very severe congenital forms that result in rapid death to milder or even asymptomatic phenotypes in adults is now described. Disease onset and/or evolution are frequently exacerbated by stress induced neurological deterioration resulting in death within 2-5 years after the first neurological signs. The specific susceptibility of WM could result from the unique regulation of eIF2B and/or the increased stress vulnerability of specific brain cell types. Abnormalities of glial cells such as proliferation of immature oligodendrocytes contrasting with paucity /dystrophic astrocytes have been found. We developed an inducible knock out (cKO) mouse model for eif2b using CreERT2 system. The gene conditional deletion is specifically induced in radial glial cells and astrocytes thanks to a CRE recombinase under the control of the Glast promoter, while the temporal induction is obtained with 5 days injection of tamoxifen in 2 months old female mice. Mice are analyzed ten days and five weeks after induction of deletion. After five weeks of cKO Eif2b induction, mice exhibited clinical symptoms with progressive lower limb paralysis leading to dramatic weight loss in one week leading to death. Immunohistochemical analysis of the brain of treated females showed an absence of myelin loss or white /gray matter vacuolization. A proliferation dysregulation of progenitor cells was observed in the corpus callosum (CC) and hippocampus in the presymptomatic mice brain (10 days treatment). This dysregulation was more important in the five weeks treated mice. Significant decrease in the surface of GFAP expression as well as in the number of oligodendrocytes was observed. In contrast we observed an increase in the number of oligodendrocyte precursor cells.

These results show that inhibition of Eif2b expression in the glial cell line in the female adult mouse brain leads to histological and phenotypic effects that mimic the signs observed in CACH/VWM patients. Our model represent so a relevant mouse model for potential therapy studies for LKDs.

T08-02B

Opposing effects of a toll-like receptor 9 antagonist on spinal cord neuronal viability through direct versus astrocyte-mediated actions

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Question: Toll-like receptors (TLRs) are primarily known for the activation of the innate immune system in response to pathogens. However, they are also expressed in the central nervous system (CNS) and have been implicated in CNS injury. In fact, earlier investigations in our laboratory indicated that intrathecal administration of a TLR9 antagonist, CpG ODN 2088, improves the outcomes of spinal cord injury. Although TLR9 is expressed in spinal cord (SC) neurons and astrocytes, the direct effects of the TLR9 antagonist on these cells have not been defined. The current studies were undertaken to investigate how TLR9 antagonism impacts SC neurons, astroglia and astrocyte-neuron interactions.

Methods: Pure neuronal cultures were established from the SC of mouse embryos. Astroglia were isolated from mixed glial cultures derived from the SC of neonatal mice. The transwell system was used for astrocyte-neuron co-cultures. Label-free nLC-MS/MS-based proteome analysis was utilized to delineate the differential proteome profile in neurons treated with CpG ODN 2088. Neuronal viability was assessed by counting β-III tubulin immunoreactive cells.

Results: We corroborated that TLR9 mRNA is expressed in SC neuron and astroglia, *in vitro.* Treatment of neuronal cultures with CpG ODN 2088 (1μM) for 24h altered the proteome profile: 202 differentially regulated proteins were identified (>1.5-fold change compared to vehicle-treated neurons). Moreover, CpG ODN 2088 protected SC neurons from kainic acid-induced excitotoxic death. CpG ODN 2088 did not rescue $TLR9^{-/-}$ neurons from excitotoxicity, confirming the dependence of the protective effect on TLR9. In contrast, the viability of SC neurons maintained in conditioned medium (CM) of CpG ODN 2088-treated astrocytes was significantly lower than those exposed to CM of vehicle-treated astrocytes. CpG ODN 2088 also reduced neuronal survival in astrocyte-neuron co-cultures. The negative effects of CpG ODN 2088-treated astrocytes on neuronal survival were not observed when $TLR9^{-/-}$ astrocytes were used. CpG ODN 2088 significantly reduced the constitutive release of CXCL1 and IL-6 by astrocytes, at 24 and 48h post-treatment.

Conclusions: These findings indicate that TLR9 antagonism alters neuronal and astroglial function as evidenced by the changes in neuronal proteome profiles and constitutive cytokine release by astrocytes, respectively. CpG ODN 2088 exerts neuroprotective effects through direct actions on neurons and, paradoxically, reduces neuronal survival via astrocyte-mediated effects.

T08-03B

A differential astrocyte reactivity is induced by omega-3 fatty acid deficiency in nuclei of rat basal ganglia

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Omega-3 fatty acid (n-3) deficiency for two generations induces oxidative stress and neurodegeneration in the rat nigrostriatal system. To understand whether impairment in glial cell function contributes to this effect, this study assessed astrocyte morpho-functional parameters in both substantia nigra and striatum of young and adult rats. Animals were divided into two groups according to the diet, supplied from mating and over two generations. The lipid source of control diet was soybean and the n-3 deficient diet was coconut oil. Glutamine synthetase activity, GFAP isoforms expression, morphological changes and number of $GFAP^{+}$ astrocytes were analyzed. The results showed a significant increase in glutamine synthetase activity in the striatum of n-3 restricted young (2.5 fold) and adult (2.0 fold) animals in comparison to the control. In the substantia nigra, this increase was observed only at adulthood (4.0 fold) and astrocytes showed an increased arborization area; whereas in the striatum, a higher fractal dimension was also found at both ages. Lower number of astrocytes was found in the substantia nigra of n-3 deficient young (40% less) and adult (20% less) animals compared to control. The expression profile of GFAP isoforms showed that n-3 deficiency was associated with a decreased expression of the phosphorylated 50 kDa band, especially in the midbrain, where additional isoforms were detected through adulthood. The results show that the

impact of n-3 dietary restriction on astrocytes is region specific within basal ganglia and indicate that their dysfunction in the substantia nigra may contribute to worsen neurodegeneration in this nucleus.

T08-04B

Mouse embryo dorsal root ganglia neuron survival was decreased in the absence of microglia

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Developmental cell death (DCD) of neurons is required for correct formation of the central nervous system (CNS). This sets up a selective process in which neurons compete for target-derived trophic signals. During embryonic development, dorsal root ganglia (DRG) neurons are generated in excess. DCD of DRG neurons, begins when sensory neuron afferents reach the embryonic mouse spinal cord (SC) (at 11.5-12.5 days of embryonic age E11.5-E12.5). DRG neuron survival during development depends on neurotrophic factors released at specific developmental stages including NGF, NT-3 and BDNF. There is now increasing evidence that DCD could be regulated by microglia. Microglia are the immune cells of the CNS. They secrete a wide range of factors, some of which can be detrimental by actively triggering apoptosis, while others, such as neurotrophic factors, that are beneficial for neuron survival. These immune cells invade the CNS at early developmental stages in the embryo. However, the exact functions of microglia during early DCD remain a mater of debate. They can have either detrimental or beneficial functions depending on the experimental conditions (in vitro or in vivo) and/or on the CNS area studied. In the embryonic mouse SC, microglia invade the parenchyma at the onset of DRG neuron DCD (from E12.5) and interact with axon terminals of dying DRG neurons localized in the dorsolateral funiculi. This finding suggests that microglia could be an active partner of developing DRG neurons during their developmental processes. In this context, we explored the role of microglia-neuron interactions during the initial DCD of DRG neurons in the SC of mouse embryos. For this study, we used two models of microglia ablation; the PU.1-/- mouse embryos, a transgenic mouse lacking microglia, and an immunological ablation of embryonic microglia in vivo. We discovered that the absence of microglia was associated with a dramatic increase in the numbers of dying DRG neurons between E12.5 and E14.5. DCD of DRG neurons expressing TrkB and TrkC was increased in the absence of microglia compared to DCD of DRG neurons expressing TrkA. We also found that activated microglia (Mac2 staining) apposed to terminals of dying DRG neurons, specifically express BDNF and NT3. These results allow us to postulate that microglia actively help embryonic DRG neuronal subclasses to survive during embryonic development.

T08-05B

How does neuronal activity regulate the formation and function of myelinated axons *in vivo*?

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In the central nervous system, the presence of myelin sheaths on axons allows rapid and accurate nerve impulse conduction that can be regulated to fine-tune neural circuit function. There is increasing evidence that neuronal activity itself can modulate myelination. During development, social isolation of juvenile mice affects their CNS myelination and in humans, repetitive training of a specific task leads to an increase myelination of the associated specific area. However, how neuronal activity precisely regulates myelination during oligodendrocyte development *in vivo* is unknown.

Zebrafish are now established as a powerful model to monitor oligodendrocyte (OL) maturation *in vivo*. In this project, we have recorded the functional activity of OLs using the genetically encoded calcium indicator GCaMP6s. We have observed calcium waves along the nascent myelin sheaths of immature OL and also along longer myelin sheath of more mature OL. This highlights that myelin sheaths are

locally "active" at distinct stages of myelination, and it suggests that functional communication between the axon and myelin sheath might regulate different steps of myelin sheath generation and growth.

Therefore, we addressed the functional contribution of neuronal activity during myelin sheath formation. By globally disrupting synaptic vesicle release using Tetanus Toxin, we have shown that inhibition of neuronal activity leads to a lower number of myelin sheath made by individual OLs (Mensch et al. accepted). Time-lapse analyses show that this due to a reduction of initial myelin sheath generation.

In addition, to determine the functional significance of neuronal activity at the later stage of myelin sheath elongation, we are currently characterising myelination along individual axons and assessing the consequence of inhibition of synaptic vesicle release. This will allow us to determine which parameter(s) of myelination (number, position, length, thickness of sheaths) are dependent of neuronal activity. We are also establishing optogenetic strategies to modulate neuronal activity in a precisely controlled manner over time and test how this affects CNS myelination *in vivo*. This work will help our understanding of how a neuron can influence signal propagation along its own axon.

T08-06B

Glial cells in the enteric nervous system are sensitive to synaptic and non-synaptic neuronal activity

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The enteric nervous system is a network of neurons and glia within the wall of the gastrointestinal tract that is able to control many aspects of digestive function independently from the brain. Enteric glial cells are closely associated with enteric neurons and their processes both within and outside enteric ganglia. Similar to other parts of the nervous system, there is communication between enteric neurons and glia. Several studies have shown that in the enteric nervous system this communication is mediated via purinergic signaling. To further unravel the crosstalk between enteric neurons and glia we use conditional transgenic reporter mice to express the genetically-encoded Ca^{2+} indicator GCaMP3 in enteric glial cells selectively. In freshly dissected preparations isolated from both adult and early postnatal (P10) mice, activation of enteric neurons induced by trains of electric pulses transmitted via a focal electrode positioned on interganglionic nerve strands induced transient increases in GCaMP3 fluorescence spreading throughout the ganglionic glial network. This confirms previous reports using synthetic Ca^{2+} indicator dyes and indicates that enteric neuron-to-glia communication is functional shortly after birth. Moreover, our system of glia-restricted Ca^{2+} reporter expression clearly reveals the potential importance of Ca^{2+} transients isolated in enteric glial cell bodies or processes. Because of the intimate association between enteric neuronal cell bodies and ganglionic enteric glia we further investigated their functional relationship by stimulating single enteric neurons through UV-mediated photolysis of the Ca^{2+} chelator nitrophenyl EGTA-AM. Neuronal photo-stimulation also induced increases in intracellular Ca^{2+} in glial cells at both stages but in this case Ca^{2+} transients were only detected in the enteric glial cells enwrapping the stimulated neuronal cell body. On average 3 to 4 enteric glial cells responded per stimulated enteric neuron, and these Ca^{2+} responses appear not to depend on synaptic activity and/or purinergic signaling mechanisms. Thus, our experiments indicate that besides monitoring synaptic neuronal activity, ganglionic enteric glial cells also respond to non-synaptic neuronal activity. Our future studies aim to identify the mediators and mechanisms involved.

T08-07B

Functional GABA-A receptors in Schwann Cells are cross-regulated in GABA-B Null mice

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Recent evidence demonstrates that Schwann cells (SCs) synthesise γ-amino butyric acid (GABA) and express its receptors, the ionotropic GABA-A and the metabotropic GABA-B. Both receptors play important roles in SCs biology. GABA-A activation leads to an increase in GABA synthesis, myelin protein expression and SC proliferation, also modulating GABA-B expression. GABA-B activation, indeed, decreases proliferation and myelin protein expression, playing a role in SC differentiation toward the state of non-myelinating SCs. Our recent studies on conditional knockout mice, specifically lacking GABA-B1 receptor in SCs (P0-CRE/GABA-$B1_{fl/fl}$ mice), confirmed alterations in the myelination process and revealed that these mice are hyperalgesic and allodynic. Therefore, both receptors are involved in SC biological processes and may cross-regulate each another. In an attempt to corroborate this hypothesis, the expression levels of different GABA-A subunits (α2-3, β1-3, α4, α5, γ2, δ) in SCs and dorsal root ganglia (DRG) neurons of conditional P0-CRE/GABA-$B1_{fl/fl}$ mice are being analysed. Preliminary data by qRT-PCR analysis revealed that GABA-A subunit expression was changed in SCs and in DRG neurons respectively. Similar results were obtained also ex vivo, in sciatic nerves and DRG of P0-CRE/GABA-$B1_{fl/fl}$ conditional mice. GABA-A activation in SCs was further supported by Ca^{++}-imaging experiments on rat SC primary cultures assessing the ability of GABA and specific ligands (e.g the agonist muscimol or the antagonist bicuculline) to change intracellular Ca^{++} concentration. Overall, our data add further evidence to the capability of GABA-A and GABA-B receptors to cross-interact, and provides a new interpretation of the intracellular events underlying GABAergic modulation in SCs and in the PNS. (*Supported by Association Francaise Contre Les Myopathies grant n° 2012/16342*).

T08-08B

Purinergic $P2Y_2$ receptors on satellite glial cells as new potential targets for the pharmacological control of trigeminal sensitization

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Sensitization of neurons in the trigeminal ganglion (TG) is at the basis of various painful conditions of the head and facial districts, often lacking a satisfactory pharmacological control. TG neuronal functions are crucially modulated by surrounding resident glial cells, the so-called satellite glial cells (SGCs), which react to painful conditions by releasing pro-inflammatory and pro-algogenic mediators and signalling molecules, thus contributing to neuronal sensitization and to the development of pain. Innovative targets for the development of more effective analgesics could emerge from a better understanding of the molecular cross-talk between TG neurons and SGCs. We focused on the role of the purinergic signalling, and demonstrated that G protein-coupled P2Y purinergic receptors (P2YRs) activated by extracellular nucleotides and expressed by SGCs are upregulated *in vitro* by pro-algogenic molecules, such as bradykinin (BK) and CGRP *(Ceruti et al., 2011, J Neurosci 31:3638-3649)*.

Here, we have first identified the P2YR subtypes specifically modulated by algogenic conditions *in vitro* as the $P2Y_1R$ (activated by ADP) and the $P2Y_2R$ (activated by UTP), and demonstrated the contribution of prostaglandins to their upregulation. Next, we have translated our data to an *in vivo* model of TG pain (namely, the injection of Complete Freund's adjuvant in the temporomandibular joint of rats; *Villa et al., 2010, Mol Pain 6:89*), and demonstrated development of mechanical allodynia, activation of SGCs, and upregulation of $P2Y_1R$ and $P2Y_2R$ expression in the ipsi-lateral TG. To unequivocally link P2YRs to the development of facial allodynia, we treated animals with PPADS (non-selective P2XR/P2YR antagonist), MRS2179 (selective $P2Y_1R$ antagonist), AR-C118925 (selective $P2Y_2R$ antagonist, from Astra Zeneca), Sumatriptan (anti-migraine agent) or the anti-inflammatory drug acetylsalicylic acid (ASA). The AR-C118925 compound completely inhibited SGCs activation, exerted a potent anti-allodynic effect that lasted over time, and was still effective when its

administration started 6 days post induction of allodynia, i.e. under sub-chronic pain conditions. Conversely, MRS2179 had no effect on facial allodynia. Similarly to ASA and Sumatriptan, PPADS was only partially effective, and completely lost its activity under sub-chronic conditions (*Magni et al., 2015, Glia. DOI 10.1002/glia.22819)*. Taken together, our results highlight the $P2Y_2R$ subtype as a new potential "druggable" target for the successful management of TG-related pain.

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T08-09B

Glial abnormalities parallel neuronal impairment in human enteric nervous system

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Background: The enteric nervous system (ENS), also referred to as the brain in the gut, contains an important number of glial cells (EGC) which are regarded to as the astrocytes in the brain. EGC are tightly packed with neurons to provide structural and functional support to them. If triggered by damaging insults, glial cells react and proliferate, a phenomenon called reactive gliosis. Although it is well established that glial cells are key players in several gut diseases, there is no data on the link between neuronal functions and EGC in the human gut. We analyzed glial morphology and its link with neuronal functioning in the submucous plexus of the human gut in health and disease.

Methods: Duodenal biopsies were taken from healthy subjects (HS) and patients with intestinal functional disorders. The submucous plexus was isolated and was used to examine neuronal functioning (calcium imaging) and ganglionic architecture by immunohistochemical stainings for S100 to label glia and HuCD to label neurons. The ratio of HuCD labeling intensity between nucleus and cytosol ratio was calculated. Volumetric measurements of S100 in the ganglia were performed on deconvolved confocal recordings. The total glial volume was calculated and expressed relative to the number of neurons.

Results: Neuronal functioning was impaired in the submucous plexus of patients, as shown by the decreased amplitude of their depolarization induced calcium transients, compared to HS ($p<0.01$). Ganglionic architecture, as revealed by glial S100 protein and neuronal marker HuCD, showed signs of gliosis and neuronal abnormalities in the submucous plexus of patients. We found a more explicit nuclear HuCD staining in submucous neurons of diseased patients (66.5±5.3% vs. 41.0±4.2%, $p<0.01$) and S100 labeling resulted to be more abundant in patients than in HS, as shown by the total glial volume measurement (27224±2214.7 vs. 3659.8±272.3 μm^3, $p<0.01$). As well, the glia volume per neuron was significantly higher in patients compared to HS (1993±382.4 vs. 362.1±139.8 μm3, $p<0.01$).

Conclusions: We show that parallel morphological abnormalities occur in EGC and neurons in pathological situations like functional intestinal disorders, even though these alterations remained undetected until now. Further evaluation of the functional impairment of EGC in the presence of neuronal abnormalities are warranted to better understand the intimate connection between these cell populations.

T08-10B

mGluR5-mediated calcium signalling in rat cortical primary astrocytes is modulated by adenosine A1 and A2A receptors

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Astrocytes play an active role in the tripartite synapse (Perea et al, 2005), as they participate in the uptake of neurotransmitters, release of gliotransmitters and express neurotransmitter receptors in their membranes (Halassa et al, 2006). The activation of the glutamate metabotropic receptor type 5

(mGluR5) in cortical primary astrocytes leads to an increase of the intracellular calcium concentrations ($[Ca^{2+}]_i$) (Baláz et al, 1997). Adenosine as neuromodulator, influences neural and glial functions and neuron-glia signaling (Abbracchio et al.,2008). Thus, we aim to evaluate the role of adenosine receptors in the modulation of calcium signalling induced by the activation of mGluR5 and to assess the cross-talk between adenosine receptors in this modulation.

Variations in $[Ca^{2+}]_i$ on Sprague Dawley rat cortical primary astrocytes were detected by Ca^{2+} imaging technique using fura 2-acetoxymethyl ester (Fura 2AM, 5 mM) at room temperature (22ºC). Cells were locally and briefly (2 seconds) stimulated with DHPG (10μM), a selective mGluR5 agonist, and the amplitude of fluorescence signals was measured in the presence of different drugs.

Using Ca^{2+} imaging technique, we observed that activation of adenosine A1 receptor (A1R)decreases the amplitude of the calcium transient induced by mGluR5 activation ($n=4$, $p<0.001$). On the other hand, activation of adenosine receptor A2A (A2AR), did not have any effect *per se*, although, the activation of A2AR is required for A1R effects since A2AR blocked, with the selective A2AR antagonist, SCH58261, reversed the A1R effect on the calcium transient amplitude ($n=5$, $p< 0,001$).

GABA transport on Sprague Dawley rat cortical primary astrocytes was initiated by addition of 30 mM [^{3}H]GABA (specific activity 0.141 Ci/mmol). Aliquots were used for scintillation counting. GAT-1 and GAT-3 mediated GABA uptake was taken as the difference between the [^{3}H]GABA uptake in the absence and in the presence of the GAT-1 or the GAT-3 blocker, respectively.

Once metabotropic receptors of ATP are able to modulate GAT-1 and GAT-3 transporters (GATs) activity (Jacob P. et al, 2014), we next evaluate the effect of mGluR5 activation upon GATs. Activation of mGluR5 decreases GABA uptake mediated by GATs in a calcium dependent way ($n=4$, $p< 0.05$).

This data suggest that adenosine modulates the calcium transients induced by mGluR5 activation in rat cortical astrocytes and that the $[Ca^{2+}]_i$ increase induced by mGluR5 activation may have an influence in GABA uptake.

T08-12B

Neuronal alarmin IL-1α evokes astrocyte-mediated protective signals against oxaliplatin neurotoxicity

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Question: Neuropathic pain is associated with glia activation, and glial inhibitors has been proposed as pain reliever. On the other hand, glial cells promote neuroprotective mechanisms leading to recovery following nervous injury. The distinction between glia painful and protective pathways is unclear and the possibility to finely modulate the system is lacking. Focusing on the initial phases of CNS alterations we studied the role of interleukin 1α (IL-1α), an alarmin belonging to the larger family of damage-associated molecular patterns (DAMPs) endogenously secreted to restore homeostasis.

Methods: Primary rat neuron and astrocyte cell cultures were obtained and treated with the anticancer agent oxaliplatin able to induce in vivo a peripheral neuropathy.

Results: Oxaliplatin induced apoptosis (caspase 3 activation) and cell viability decrease in neurons and, with less potency, in astrocytes. One μM oxaliplatin (48h) was chosen as treatment able to damage neurons but to maintain astrocytes alive. In this condition both neurons and glia released IL-1α in the culture medium. Neuronal damage was unmodified when a neuron-glia transwell co-culture was exposed to increasing concentration of oxaliplatin for 48h, whereas IL-1α release increased in a concentration-dependent manner. To evaluate the role of the cytokine we silenced IL-1α in neurons by specific small interfering RNA. IL-1α-knock down neurons co-cultured with astrocytes were more prone to neurotoxicity since 1 μM oxaliplatin increased cell mortality by about 50% in comparison to wild type neurons. On the other hand, the extracellular levels of ATP, a key neurotransmitter in neuropathic processes, strongly increased in IL-1α-deficient neuron/astrocyte co-culture treated with oxaliplatin. Moreover, in the absence of neuronal IL-1α the protective cytokine TGFβ1 was significantly

decreased in the co-culture medium. TGFβ1 release was higher from astrocytes than neurons and was reduced by the astrocyte inhibitor fluorocitrate, suggesting astroglia as target of the alarm raised by neurons.

Conclusion: Oxaliplatin induces neuronal damage provoking IL-1α release. IL-1α-stimulated astrocytes are able to protect neurons by releasing TGFβ1 and limiting ATP levels in the extracellular environment.

T08-14B

Astroglial networks modulation of bursting activity dynamics

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Dynamic interactions between astrocytes and neurons play crucial roles in the regulation of brain network activity. Astrocytes can directly modulate neuronal excitability and synaptic plasticity and regulate rhythm generation and neuronal network firing. Their key feature is the ability to form plastic and extensive networks through gap junction channels. It has recently been shown that astroglial networks limit neuronal network activity. However, it is currently unclear how astroglial networks influence neuronal excitability and participate in synchronizing neuronal populations. To investigate how astroglial networks regulate neuronal network activity, we performed electrophysiological recordings in hippocampal slices from mice with disconnected astrocytes, in which both astroglial gap junction forming proteins, connexin 30 (Cx30) and connexin 43 (Cx43), are knocked out. Synchronized discharges were recorded in an acute pharmacological model of epileptic-like activity. We observed that the frequency of bursting activity in Cx knockout mice was drastically increased, while the duration of neuronal depolarizing bursts was severely reduced. Furthermore, pyramidal neurons were more depolarized due to an enhancement of synaptic background activity. This suggests that increased synaptic bombardment promoting resting membrane potential depolarization increases excitability, thus facilitating triggering of neuronal bursts, but compromises the strength of synchronized events, by a decreased neuronal release probability. To investigate local dynamics of bursts generation, we performed multielectrode array recordings in the CA3 area of the hippocampus. We found that bursting activity in the CA3 region was more focal in Cx KO mice, indicating that the number of neurons recruited was decreased. Altogether, these results indicate that gap junction-mediated astroglial networks promote neuronal coordination during synchronized events.

T08-15B

Dynamics of ionic shifts in cortical spreading depression

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Cortical spreading depression is a slowly propagating wave of near-complete depolarization of brain cells followed by temporary suppression of neuronal activity. Accumulating evidence indicates that cortical spreading depression u*nderlies the migraine aura and that similar* waves promote tissue damage in stroke, trauma, and hemorrhage. Cortical spreading depression is characterized by neuronal swelling, profound elevation of extracellular potassium and glutamate, multiphasic blood flow changes, and drop in tissue oxygen tension. The slow speed of the cortical spreading depression wave implies that it is mediated by diffusion of a chemical substance, yet the identity of this substance and the pathway it follows are unknown. Intercellular spread between gap junction-coupled neurons or glial cells and interstitial diffusion of K^+ or glutamate have been proposed. Here we use extracellular direct current potential recordings, K^+-sensitive microelectrodes, and two-photon imaging with ultrasensitive Ca^{2+} and glutamate fluorescent probes to elucidate the spatiotemporal dynamics of ionic shifts associated with the propagation of cortical spreading depression in the visual cortex of adult living mice. Our data argue against intercellular spread of Ca^{2+} carrying the cortical spreading

depression wavefront and are in favor of interstitial K^+ diffusion, rather than glutamate diffusion, as the leading event in cortical spreading depression.

T08-16B

Neurofilaments enter in oligodendrocytes via clathrin-dependent endocytosis to promote their growth and survival in vitro

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Neurofilaments (NF) are found in the cerebrospinal fluid (CSF) during multiple sclerosis (MS), but their role outside the axon is unclear yet. We previously showed that, depending on the step of their purification, NF fractions increase in-vitro oligodendrocyte (OL) progenitor proliferation and/or differentiation (Fressinaud et al., 2012). However their cell-entry molecular mechanism is unknown. Using rat OL secondary cultures we localized NF by double immunocytochemistry and confocal microscopy. OL cultures grown in chemically-defined medium were treated with NFP2 (2nd pellet of the purification) or P5 fractions (5th pellet) for 24 h, and NFP2 were localized with anti-NFH antibody in cytoplasmic processes of myelin basic protein (MBP+) expressing OL, whereas NFP5 were undetectable. This uptake was further confirmed and characterized, using dynasore, an inhibitor of dynamin (involved in clathrin-dependent endocytosis), which inhibited by 50% the incorporation of NFP2 fractions into OL. Thus clathrin-dependent endocytosis is partially responsible for internalization of NF. Both OL progenitors and mature OL were shown to take NFP2, in agreement with the biological activity of NF promoting OL proliferation and maturation. In addition, we observed that TRITC-labelled tubulin (TUB*) appeared to localize in MBP+ OL (Fressinaud et al., 2012). Confocal microscopy confirmed the labelling of OL progenitors (A2B5+ cells) as well as mature OL (MBP+ cells) by TUB*. Nevertheless and contrary to NF, dynasore did not inhibit TUB* uptake, suggesting that its endocytosis is clathrin-independent. This study confirms that axon cytoskeleton proteins can be internalized in OL by several mechanisms. This process could be involved during demyelination, and release of axon proteins might favourize remyelination.

T08-17B

Role of the alpha-secretase TACE in Central Nervous System myelination

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The ADAM family of proteins belongs to the zinc family of proteases that are involved in the ectodomain shedding of several growth factors. We previously demonstrated a key role for the α-secretase TACE in Peripheral Nervous System (PNS) myelination by TACE-mediated cleavage and subsequent inhibition of Neuregulin1 (NRG1) type III activity. Unlike the PNS, in which NRG1 type III is an essential instructive signal for myelination, in the Central Nervous System (CNS) oligodendrocyte (OL) development and myelination are likely controlled by several growth factors some of which undergo cleavage by secretases.

To assess whether TACE plays a similar role in PNS and CNS myelination, we investigated its role *in vitro* and *in vivo* in OL development and myelination. *In vitro* experiments showed that immunopanned A2B5+ oligodendrocyte precursor cells (OPCs) grown in conditioned medium from TACE-null DRG neurons undergo marked apoptosis. Further, when cocultured, wild type OPCs poorly myelinate TACE-null DRG neurons, suggesting that neuronal TACE is required for *in vitro* myelination. In agreement, transgenic mice lacking TACE in CNS neurons are hypomyelinated and have an aberrant myelin sheet throughout development. On the contrary, specific ablation of TACE in OLs *in vivo* did not affect developmental myelination.

Our data strongly suggest that the neuronal α-secretase TACE is required for proper CNS myelination. Further, our studies confirm that secretases are important post-translational regulators of myelination although the mechanisms controlling CNS and PNS myelination are distinct.

T08-18B

Age-related cognitive impact in a transgenic model of astrocytic dysfunction

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Cognitive ageing, including impairment of spatial learning and memory is one of the widespread effects of brain ageing that affects approximately half of the population over 60 years of age. Astrocytes possess unique morphologic and phenotypic features that allow them to monitor their neighbourhood and dynamically respond to changes. Moreover, several studies report that astrocytes increase their number about 20% in response to injured or damaged neurons during ageing, possibly to provide the same level of neuroprotection that is present in the brain of a young animal. Furthermore, astrocytes also have an important role in age-related excitotoxicity mechanisms, through glutamate clearance by GLAST and GLT-1 transporters. However, the mechanisms underlying neuron-glia interactions in cognitive ageing are not fully understood. Considering that the impairment of astrocytic function might have implications in neurotransmission, metabolism and brain homeostasis via exocytotic release of substances, we aim at understanding the impact of astrocytic-exocytosis in age-related cognitive decline. For that, we will use dnSNARE mouse model of exocytosis impairment obtained by conditional blockade of vesicular release specifically in astrocytes. The expression of transgenes was confirmed to be restricted to GFAP- and S100B-positive astrocytes. Cognitive function was assessed by the Morris Water Maze and molecular correlates were sought in brain tissue of 20 months-old dnSNARE and respective littermate controls.

T08-19B

An astrocyte-dependent mechanism that links increased TNFα levels to a persistent change of function in cognitive circuits: relevance to multiple sclerosis

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Work of the last 15 years has shown that the cytokine TNFα exerts a physiological control on hippocampal synaptic transmission and plasticity (Beattie et al., Science, 2002; Stellwagen and Malenka, Nature, 2006), including via astrocyte-dependent glutamate release (Bezzi et al., Nat Neurosci., 2001; Jourdain et al., Nat Neurosci, 2007; Santello et al., Neuron, 2011). Here we investigated the synaptic effect of TNFα at high concentration (10-fold the constitutive one), to mimic the enhanced cytokine levels in pathological conditions. We show that high TNFα induces slow and persistent modification of excitatory transmission (mEPSC frequency) at dentate granule cell synapses via activation of pre-synaptic NMDAR. By using $TNFR1^{-/-}$ mice, we also show that the cytokine effect requires TNFR1 signaling, and by knocking-down conditionally TNFR1 in GFAP-positive cells, that such signaling takes place in astrocytes. To ascertain if the above TNFα-dependent synaptic alteration occurs in a specific pathological condition and contributes to disease pathogenesis, we focused on Multiple Sclerosis (MS). Thus, half of the MS patients suffer from cognitive impairment related to hippocampal dysfunction (Benedict and Zivadinov, Nat Rev Neurol, 2011); moreover TNFα signaling via TNFR1 is clearly implicated in both human MS (Gregory et al., Nature, 2012) and in murine models

of the pathology, such as Experimental Auto-immune Encephalomyelitis (EAE) (Probert et al., Brain, 2000). We found that TNFα levels in EAE are increased in the hippocampus, specifically in the dorsal region, at the border with the third ventricle, associated with parenchymal inflammation and infiltration. Such TNFα increase induces persistent synaptic changes at granule cell synapses similar to those observed upon exogenous TNFα application. We demonstrate that the synaptic changes depend on astrocyte TNFR1 signaling, as they were not observed in TNFR1$^{-/-}$ mice, but were largely reconstituted upon conditional re-expression of TNFR1 solely in astrocytes. Finally, EAE animals showed impaired contextual memory in the fear conditioning test. This form of memory requires intact function of the dorsal hippocampus excitatory circuitry, suggesting that the identified astrocyte mechanism may play a pathogenic role in the cognitive disturbances observed in MS patients. This research was supported by Swiss National Science Foundation NCCR “Synapsy”, grant 31003A-140999 and ERC Advanced grant 340368 “Astromnesis” to AV.

T08-20B

Nanofiber-platform for human pluripotent stem cell -derived neural cells

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Question: Modelling central nervous system processes and deficits *in vitro* is currently based on the wide use of 2D cell culture models. However, 2D models lack the structural architecture that mimic the natural environment of cells. Thus, more 3D-like culture conditions are needed to provide biologically more relevant environment for cells. Synthetic nanofibers are one option for this kind of cell culture models.The benefits of nanofibers include their assembly into a variety of architectures by manipulating their alignment, stacking and folding. In addition, they are easy to functionalize through encapsulation or attachment of bioactive components such as extracellular matrix proteins and growth factors. Previously, nanofiber platforms have shown to be compatible with murine-derived neural cells but only few studies with any type of human-derived neural cells have been performed.

The aim of this study is to test the suitability of aligned nanofibers for *in vitro* modelling of cellular interactions in central nervous system using human pluripotent stem cell -derived neurons, astrocytes and oligodendrocyte precursor cells (OPCs).

Methods: Commercially available, aligned polycaprolactone nanofibers (Nanofiber Solutions) were used as a platform and cells were seeded on top of the fibers. Extracellular matrix molecule -coating on nanofibers was also studied. The cytocompatibility of the fibers and the attachment, growth and differentiation of the cells were studied using immunocytochemistry and imaging analysis. In addition, more detailed analysis of thebehaviour of OPCs on nanofibers was performed using electron microscopy.

Results: Nanofibers were non-cytotoxic and immunocytochemical staining confirmed the attachment and viability of all the tested cell types on the nanofibers. All the cell types also followed the fiber alignment. OPCs contacted the plain nanofibers most prominently and did not require additional protein coating. Growth of neurons and astrocytes on fibers could be considerably enhanced by coating the fibers with extracellular matrix molecules.

Conclusions: Nanofibers can be used as a cell culture platform for human pluripotent stem cell-derived neurons, astrocytes and oligodendrocytes.

T08-21B

Generation of astrocytes from human induced pluripotent stem cells to investigate astrocyte biology in neurodegenerative diseases

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For more than 100 years the neuron was accepted as the most important cell type for cognitive function in the brain. Astrocytes were discussed to have only supportive functions in the central nervous system “filling the gap” between neurons. However, there is growing evidence that the contribution of astrocytes is far more complex and critical for brain function in physiology and pathology. Astrocytes are important regulators of brain homeostasis metabolism and synaptic activity thus play a critical role to achieve normal brain function. Astrocyte dysfunction is also indicated to be critical in neurodegenerative disorders like Alzheimer´s Disease (AD). A better understanding of astrocyte biology and their interplay with neurons will help to develop disease modifying treatments for these conditions. In this study, we differentiated mature human astrocytes from control and AD-patient derived iPSCs to study their role in disease in greater detail.

To uncover the potential role of human astrocytes in synaptic maturation hiPSC-derived astrocytes were used in co-culture experiments with hiPSC-derived neurons. In addition the extracellular matrix (ECM) of these cells was used as a substrate for hiPSC-derived neurons in the same context. Synaptic marker expression and excitotoxicity were measured to assess neuronal maturity. Both, direct co-culture as well as astrocyte-derived ECM lead to increased neuronal survival. Furthermore, astrocyte ECM had a proliferative effect on neuronal precursor cells. In a next step we successfully differentiated human astrocytes from AD-patient derived hiPSCs. Immunofluorescence staining and qPCR revealed the expression of astrocyte markers (e.g. GFAP, S100ß).

Using a next generation sequencing approach we aim to identify novel disease relevant genes/ pathways in hiPSC-derived astrocytes from AD patients. These cells will also be used to build *in vitro* models for AD dissecting their role in the disease process, ultimately identifying patient-relevant astrocyte pathology.

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T08-22B

Astrocytes gate synaptic transmission from unmyelinated sensory afferents

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Sensory inflow continuously bombards the central nervous system. The most relevant information for a given context is selected early on by filtering incoming signals at the first synapse. In the spinal cord this is accomplished by GABA mediated presynaptic inhibition of primary afferent terminals. Despite intense research, the identity of the cells that release GABA onto primary afferents remains unknown. Here we show that Aδ/C sensory fiber stimulation triggers the release of GABA from nearby astrocytes, which inhibits the release of neurotransmitter from sensory afferents with a time course compatible with presynaptic inhibition. Our findings provide direct evidence for primary afferent inhibition by astrocytic GABA release and demonstrate that astrocytes compute information in the same temporal domain as neurons.

T08-23B

Metabolic modulation of mitochondria reduced glial reactivity and hyperalgesia in inflammatory and neuropathic chronic pain models

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Question: Activation of glial cells is emerging as key mechanisms underlying pain. In response to different injury stimuli, astrocytes and microglia, undergo morphological and functional changes, known as glial reactivity. Glial reactivity is associated with a reduced mitochondrial function and increased glycolysis in astrocytes. Reversing the metabolic remodeling by treatment with the dichloroacetate drug (DCA), reduces glial reactivity and the progression of neuronal death in an Amyotrophic Lateral Sclerosis animal model. This work evaluates whether glial metabolic remodeling by administration of DCA, may modulate the mechanisms involved in the generation and persistence of chronic pain.

Methods: Inflammatory pain was induced in adult adult Sprague-Dawley rats by right hindlimb intraplantar injection of complete Freund's adjuvant (CFA). Neuropathic pain model was performed by chronic constriction injury (CCI) of the sciatic nerve in adult C57BL6 mice. Animals were treated with either DCA (500g/L in drinking water) or water (control). Thermal hyperalgesia and mechanical allodynia (using von Frey filaments) were analyzed on the plantar surface of the hind limbs. After 14 days from CFA injection and 19 days from CCI the animals were sacrificed to analyze the glial response in the spinal cord by GFAP (reactive astrocytes) and Iba-1(reactive microglia) immunofluorescence. Lumbar spinal cord segments were freshly removed and hemisectioned in ipsi and contrateral to injury halves to assess high-resolution respirometry in the Oroboros oxygraph 2k.

Results: In both models DCA treatment significatively reduced the ipsilateral hyperalgesia compared to the untreated group, without affecting contralateral sensitivity. Analysis of spinal cord sections, showed decreased GFAP and Iba-1 immunofluorescence in the ipsilateral dorsal horn in DCA treated compared to the untreated animals. Moreover, DCA increased Oxygen consumption in the spinal cord compared to untreated animals.

Conclusions: These results allow us to conclude that the administration of DCA is effective in reducing hyperalgesia and spinal cord glial reactivity. DCA may offer a therapeutic alternative to contribute to chronic pain treatment where few effective pharmacological tools exist.

T08-24B

3D volume imaging of calcium dynamics in astrocytes

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Recent studies have shown that astrocytes have a complex spatiotemporal pattern of calcium dynamics underlying communications with environing cells. Indeed, not only calcium signaling in astrocytes is recruiting different spatial locations, from microdomains to extended portions of processes, which can even involve the cell body or propagate to other cells, but they also have a temporal (t) coding ranging from fast transients to prolonged elevations, either in an asynchronous or in a coordinated fashion (reviewed in Volterra et al, Nature Rev Neurosci, 15:327-335, 2014). This functional complexity parallels the morphological complexity of astrocytes, with highly ramified processes down to a sub-resolved optical scale and intrinsically tridimensional (3D) structures lacking any clear functional unit element like dendritic spines. Moreover, an individual astrocyte entertains complex and heterogeneous relations with neighboring structures including blood vessels, neuronal synapses, and other glial cells. This picture makes calcium imaging in astrocytes and its interpretation a real methodological challenge. Thus, proper understanding of the role of astrocytes requires that calcium signal investigations take into account the astrocytic 3D+t context. However, most of the studies performed to-date used two-photon laser scanning microscopy (2plsm) in a single focal plane (often about 1 µm thick and arbitrarily selected). Here, by using 2plsm with a piezoelectric actuator and an acousto-optic deflector, we imaged calcium dynamics in the entire volume of single astrocytes in adult mouse hippocampal slices at a resolution allowing visualization of the direct relation between calcium events and 3D morphology. Furthermore, we developed a dedicated framework for processing and analysis of the “big data” generated by these 3D+t multispectral acquisitions. As a result, we find that recording from single focal planes, even when the best plane is selected, does not correctly report good part of the calcium activity of an astrocyte. For example, using the plane comprising the cell body

and recording somatic calcium events clearly does not provide a correct readout of the global astrocytic activity. Indeed, we observe calcium events dissociated from the somatic events that occur asynchronously and with different characteristics in different 3D portions of the astrocyte, notably in the processes and end-feet in contact with blood vessels.
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T08-25B

Fractalkine signaling is not required for ocular dominance plasticity

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Synaptic plasticity is critical for the development and maintenance of efficient nervous system function throughout the lifespan. The mechanisms which implement functional and structural changes at synapses, however, are poorly understood. Despite being classically characterized as immune cells, microglia have recently been shown to play a role in normal brain function by restructuring and removing synapses. To understand microglial effects on synapses it is important to determine the molecular players that mediate the interaction between microglia and synaptic elements, including the signal facilitating recruitment of a microglial process to a synapse. Fractalkine, a chemokine well studied in neuroinflammatory signaling, is particularly well positioned for this role given its chemotactic signaling properties, activity-dependent regulation, and specificity of ligand and receptor expression. To characterize the role of fractalkine signaling in microglia-neuron interactions in the developing brain, we first characterized baseline microglia function in the absence of fractalkine signaling. We found no changes in the density, morphology, or motility of microglia in the primary visual cortex of mice with wildtype, heterozygous, or homozygous disruption of the fractalkine receptor, indicating no overt changes in microglial function in the absence of fractalkine signaling. To determine whether fractalkine is necessary for visual system plasticity, we used intrinsic optical signal imaging to assay functional ocular dominance plasticity in adolescent mice. We found normal plasticity in mice with both heterozygous and homozygous disruption of the fractalkine receptor as compared to wildtype controls, indicating that fractalkine is not involved in this form of plasticity. Because fractalkine has been implicated in synapse remodeling earlier in development, we analyzed retinogeniculate remodeling in the lateral geniculate nucleus, an early developmental process that is dependent on microglial phagocytosis of synapses. Again, we observed normal plasticity in mice heterozygous and homozygous for fractalkine disruption. Given recent reports showing that fractalkine is critical for the normal development of circuits throughout the brain, our findings suggest instead that the contribution of fractalkine signaling may be limited to microglia-driven remodeling of specific neuronal pathways during limited developmental periods.

T08-26B

Electrophysiological characterization of human pluripotent stem cell derived oligodendrocyte precursor cells

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Oligodendrocytes are cells which myelinate the axons of the brain. Oligodendrocyte precursor cells (OPCs) exist in the adult brain as the major proliferative cell population. Due to their proliferative and precursory nature, OPCs are interesting in the context of regeneration and remyelination. However, OPCs are not a homogenous cell population and differ in their electrophysiological properties. It has been suggested that the electrically excitable population of OPCs would preferentially myelinate functionally active axons.

In this study, our aim was to produce and characterize OPCs from human pluripotent stem cells.

For this, human pluripotent stem cells were differentiated into OPCs using previously described protocol. The oligodendroglial identity of the cells was confirmed with antibodies. The development of

electrophysiological properties of OPCs was studied with whole cell patch clamp. The differentiating cell population was found to consist of electrically active and electrically passive cells. The electrically active cells responded to current injections with abortive or full action potentials. Measured cells were collected as single cells for gene expression analysis.

T08-27B

Flavonoid hesperidin modulates synapse formation on cerebral cortex and increases the synaptogenic potential of astrocytes

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The Central Nervous System is a target for several neurodegenerative diseases characterized by neuronal death, synaptic and glial dysfunctions. Currently, new drugs have been studied as therapeutic alternatives for these diseases and, among them, flavonoids have been noted for their remarkable neuroprotective actions. However, little is known about the effects of these compounds on neuronal and glial physiology. Here we investigated the actions of the flavonoid hesperidin on synapse formation in vitro, as well as the role of astrocytes as mediators of hesperidin's actions. **Methods:** Cortical neurons and astrocytes cultures were prepared from 14-15 embryonic days and 1-2 post-natal days Swiss mice, respectively. The neuronal cultures were treated with hesperidin (5 μM) for 24 hours or astrocyte conditioned medium (ACM) for 3 hours, and analyzed for synapses formation. Signaling and secretion of TGF-β1, a synaptogenic astrocyte derived-molecule (Diniz et al., 2012; Diniz et al., 2014), were analyzed in astrocyte cultures treated by hesperidin. Moreover, we evaluated if TGF-β1 secretion by astrocytes was involved in synapse formation induced by the ACM. **Results:** Immunocytochemistry for the synaptic proteins, PSD95 and Synaptophysin, revealed that treatment of neurons with hesperidin increased by 40% the number of synapses. This event was followed by a 75% decrease in neuronal death and 85% increase in pre-synaptic activity, although we did not observe differences in the levels of the synaptic proteins. ACM increased the number of synapses by 130%, whereas ACM, from astrocytes treated by hesperidin, increased by 250%, showing that hesperidin enhances the synaptogenic potential of astrocytes. Hesperidin increases the secretion of TGF-β1 and activates its signaling in astrocytes, as noted by the increase of SMADs 2/3 nuclear translocation and phospho-SMADs 2/3 levels in these cells. Furthermore, neutralization of TGF-β1 activity of the ACM significantly reduced its synaptogenic potential. **Conclusion:** Our data indicate a new function for hesperidin on synapse formation, and suggest a new mechanism of action of this compound in synaptogenesis, through the control of soluble factors production by astrocytes. The protocol of this study was approved by the Committee for Animal Research of the Federal University of Rio de Janeiro. **Support:** CNPq, CAPES, FAPERJ, Ministério da Saúde.

T08-28B

Neuronal activity dependent regulation of CNS-precursor cells in health and disease

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Myelination of neural axons is essential for normal brain function as it ensures fast information transmission and axonal maintenance. Myelin sheaths are provided by oligodendrocytes, which originate from oligodendrocyte precursor cells (OPCs).

In many brain white matter diseases — such as multiple sclerosis — myelin is lost and even though OPCs are recruited to the sites of lesion and capable of repair, remyelination often fails, leading to physical and mental disability. In order to promote repair in white matter diseases, it is essential to understand how terminal differentiation of OPCs is regulated.

It has recently been shown that synaptic input can promote myelination and regulate proliferation of OPCs. Thus we used optogenetics as a tool to simulate synaptic input into cells *in vitro,* in order to

investigate its effect on OPCs. We found that 1Hz stimulation increased migration and decreased proliferation but promoted differentiation.

These findings prompted us to investigate synaptic input in cell lines derived from Glioblastoma Multiforme patients. These cells are characterised by aberrant proliferation and migration and in some cases show expression of the OPC-marker NG2, indicating that they might originate from OPCs. Indeed, preliminary experiments show that stimulation of these cells increases their proliferation, indicating that synaptic input might play a role in the development of gliomas.

T08-29B

Purines released from astrocytes inhibit excitatory synaptic transmission in the ventral horn of the spinal cord

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Spinal neuronal networks are essential for motor function. They continuously adapt their activity to the internal state of the organism and to the environment. This plasticity can be provided by different neuromodulators, usually thought of as being released by dedicated neurons. However, in other networks from the central nervous system, synaptic transmission is also modulated by transmitters released from astrocytes. The star-shaped glial cell responds to neurotransmitters by releasing gliotransmitters, which in turn modulate synaptic transmission. We investigated if astrocytes present in the ventral horn of the spinal cord modulate synaptic transmission. We evoked synaptic inputs in ventral horn neurons recorded in a slice preparation from the spinal cord of neonatal mice. Neurons responded to electrical stimulation by monosynaptic EPSCs. We used mice expressing eGFP under the glial fibrillary acidic protein promoter to identify astrocytes. Chelating Ca^{2+} with BAPTA in a single neighboring astrocyte increased the amplitude of synaptic currents. In contrast, when we selectively stimulated astrocytes by activating PAR-1 receptors with the peptide TFLLR, the amplitude of EPSCs evoked by a paired stimulation protocol was reduced. The paired-pulse ratio was increased, suggesting an inhibition occurring at the presynaptic side of synapses. In the presence of blockers for extracellular ectonucleotidases, TFLLR did not induce presynaptic inhibition. Puffing adenosine reproduced the effect of TFLLR and blocking adenosine A_1 receptors prevented it. Altogether our results show that ventral horn astrocytes are responsible for a tonic and a phasic inhibition of excitatory synaptic transmission by releasing ATP, which gets converted into adenosine that binds to inhibitory presynaptic A_1 receptors. For that reason we postulate that purines released from astrocytes modulate the production of movements. We are currently investigating how spinal astrocytes are activated under physiological conditions. We will next analyze how ATP is released and then determine the kinetics of purine release during locomotion. This will allow us to figure out if the release is tonic, or if it occurs in phase with rhythmic movements.

T08-30B

Membrane mobility of the astroglial glutamate transporter GLT-1

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Glutamate is the major excitatory neurotransmitter in the brain and its concentration at the synapse is tightly regulated in order to maintain precise neuronal transmission and prevent excitotoxicity caused by excessive glutamate spill over and prolonged activation of glutamate receptors. In the hippocampus 90% of extracellular glutamate is cleared via astrocytic transporters, predominantly GLT-1.

To gain an insight into GLT1 distribution and trafficking we used superecliptic pHluorin (SEP; pH-dependent form of GFP) inserted into second extracellular loop of rat GLT-1 and used this construct as a probe for monitoring distribution and mobility of surface-expressed GLT-1 in astrocytes.

The insertion of GLT-1-SEP fusion protein retain its transporter function as tested in HEK 293 cells and the construct was expressed almost entirely on the astrocyte surface in dissociated and

organotypic hippocampal cultures. Despite being overexpressed GLT-1-SEP surface distribution was not uniform and clusters could be clearly identified.

To study surface mobility of the transported we usedFluorescenceRecoveryAfterFotobleaching technique and assessed that around 75% of the transporter in mixed dissociated culture was a mobile fraction. In organotypic culture this value was lover - around 69%. The speed of the diffusion is greater than previously reported for AMPA or NMDA receptors as half time of fluorescence recovery ranges from 1.5 seconds in dissociated culture to 2.3 seconds in organotypic slices.

Glutamate application increases GLT-1 mobility and this effect can be abolished by blocking glutamate receptors or by deleting C-terminus from GLT-1 suggesting that phosphorylation or interactions with other proteins is essential for that effect.

In summary, this work reveals that GLT-1 is highly mobile protein on the surface of astrocytes and that this trafficking is regulated by synaptic transmission.

T08-31B

EphB3 regulates gliotransmission following traumatic brain injury

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Traumatic brain injury (TBI) is one of the leading causes of morbidity and mortality worldwide. It results in a number of acute pathological alterations, including ionic dysregulation and extracellular release of transmitters. Acute release of transmitters is thought to play a significant role in excitotoxic cell death; however, progressive neuronal loss and synaptic degeneration persist for long periods after TBI. Currently, our understanding of transmitter functions in sub-acute and chronic TBI is limited. Glial cells, in particular astrocytes, play an important role in maintaining synaptic integrity and function after injury by passively and actively regulating transmitter levels in the synaptic cleft. Our laboratory has shown that a family of receptor tyrosine kinases (i.e. Eph receptors) and their cognate ligands (i.e. ephrins) regulate synaptic function and formation. We hypothesize that neurons communicate with astrocytes through ephrinB3-EphB3 signaling to enhance gliotransmitter synthesis and release in the synapse, which, in turn, participates in progressive synaptic damage. We have found that EphB3 signaling can enhance D-serine release from cultured astrocytes and in the hippocampus by regulation of serine racemase activity, the enzyme responsible for conversion of L-serine to D-serine. After controlled cortical impact (CCI) injury in mice, serine racemase and D-serine levels are increased in astrocytes and reduced in pyramidal neurons. Moreover, CCI injury leads to increased total D-serine levels in the hippocampus, reduced synaptic plasticity and deficits in hippocampal-dependent learning behavior that can be ameliorated in the absence of EphB3 receptors. These findings support our hypothesis that EphB3 signaling participates in progressive synaptic damage that is potentially mediated by D-serine release from astrocytes.

T08-32B

Microglia changes in rat dorsal cochlear nucleus correlate to behavioural tinnitus evidence

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Tinnitus is a phantom auditory perception (buzzing or ringing) that can become chronical, affecting quality of life similarly to chronic pain. Although several risk factors have been identified, its etiogenesis has not been clarified yet. Dorsal cochlear nuclei (DCN) show changes in plasticity that are thought to be necessary for tinnitus onset but not after its chronicization (Brozoski et al. 2011). One of the major problems in animal tinnitus models is the unreliability of tinnitus induction protocols: a large fraction of treated animals, in fact, do not develop the symptom (Koehler and Shore 2013), and tinnitus is also often associated with deafness and/or hyperacusia, which complicate interpretation of results. After noise trauma, neural plasticity in DCN fusiform cells appears associated to actual tinnitus presence (Koehler and Shore 2013).

We investigated the possible involvement of microglia in tinnitus-associated aberrant DCN plasticity. In our experiments we induced tinnitus with unilateral cochlear destruction, noise trauma or salicylate and observed Iba-1 immunofluorescence in DCN slices by confocal microscopy.

Although all treatments were able to induce tinnitus (tested as in Turner 2006), DCN microglial responses of tinnitus-positive animals were different: after salicyilate treatment, microglia significantly increased in density (from 117.3+47.2, n=15 to 215.3+54.8 cell/mm2, n=18), but did not show activation signs, whereas after cochlear destruction, microglia showed a larger increase in density (maximal in the ipsilateral nucleus after 5 days, 466,4+185,7; n=14) and clear signs of activation. After noise trauma, microglia became less uniformly distributed, showing a cluster in the DCN region corresponding to noise trauma frequencies (n=1). Microglia activation after cochlear destruction was necessary for tinnitus onset: in animals treated with minocycline after surgery, neither microgliosis (after 5 days, ipsilateral DCN density was 144.1+33.4, n=5) nor behavioural signs of tinnitus were observed. On the other hand, salicylate-induced tinnitus was not dependent on microglia activation, since behavioural signs of tinnitus were observed after treatment with salicylate and minocycline (n=2).

Our results suggest that DCN microglia follows different functional pathways upon different tinnitus-inducing treatment. A similar or even larger heterogeneity in human tinnitus population would agree with the wide variability of different treatment efficacy.

T08-33B

Glia-to-neuron shuttling of miR-146a via extracellular microvesicles modulates synaptotagminI translation in neurons

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It is widely accepted that glial cells secrete soluble molecules that mediate homeostatic synaptic plasticity and influence neurotransmission upon injury and inflammation. An additional secretory mechanism has been recently described in glial cells, which may play a role in glia-to-neuron signalling: upon activation, astrocytes and microglia, release circular membrane fragments, known as extracellular vesicles (EVs), which contain several components of donor glia (RNAs, proteins, lipids) and may function as efficient intercellular delivery mechanism. Using miRNA real-time PCR panels, we identified a set of miRNAs differentially expressed in EVs produced by pro-inflammatory vs. pro-regenerative microglia. Among them we found miR-146a, a glial-enriched miRNA, which is altered in brain disorders and targets neuron specific genes. To investigate whether glial EVs may transfer their miR-146a cargo to neurons, cultured hippocampal neurons were transfected with a *Renilla* Luciferase-based miR-146a sensor and exposed to EVs for 24-72h. By this approach we showed that miR-146a-storing EVs but not EVs produced by astrocytes treated with the anti-miR-146a inhibitor induce a significant increase in neuronal miR-146a levels. This increase was strongly inhibited when neuron-EV interaction was prevented by clocking phosphatidyl serine residues on EVs, a determinant for EV-recognition. Finally, we found that exposure to miR-146a-storing EVs results in decreased immunoreactivity of a validated miR-146a target, i.e. the synaptic vesicle protein synaptotagmin I. Taken together, our data indicate that EVs deliver to neurons biologically active miR-146a, highlighting the capability of glial cells to modulate neuronal gene expression. In order to investigate how glial EVs transfer their miRNA cargo to neurons we took advantage of optical manipulation combined with live imaging. This approach revealed that EVs positioned on the cell body make a stable interaction with neurons, staying attached to the neuronal surface up to 1h. Together with confocal analysis of fixed neurons exposed to EVs for different time points, this observation ruled out the possibility that EVs

undergo rapid internalization or full fusion with cell membrane. Further investigation is ongoing to identify by proteomic analysis the surface proteins mediating EVs-neuron interaction and to clarify whether EVs can open a transient pore to transfer their cargo to neurons.

T08-34B

Development of co-culture platfrom for neuron-oligodendrocyte research

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Question: *In vitro* co-culture models represent an important research tool in addition to animal models since animal models show some drawbacks. Many disadvantages are linked to species differences and animal models are rarely the exact counterparts of human diseases. In addition, animal models in neuroscience include exceptionally high costs. Thus, other model systems are also needed. Traditional *in vitro* cell culture techniques do not provide a controlled environment and thus these models are limited in several ways. Microfluidics technology enables the generation of multi-chamber culture platforms that allow the segregation of different cell types and guiding cell growth. In addition, the environment of segregated cell regions can be manipulated. Microfluidics-based co-culture models for neuron-oligodendrocyte research exist but yet lack important features. For example, myelination event is still challenging to detect in these models. The aim of this study was to develop a co-culture platform which contains more controlled culture conditions to study myelination.

Methods: Microfluidic co-culture platform is manufactured from polydimethylsiloxane (PDMS). The platform contains chambers for neurons and oligodendrocytes and microchannels to guide and restrict the growth of cells. The platform is designed to enable multiple measurement methods including electrophysiological approaches. Human embryonic stem cell (hESC) -derived neurons and oligodendrocytes are used as cell components in the platform. Cell culture and differentiation are performed according to the routine methods developed in our laboratory.

Results: Co-culture platform can be successfully and repeatedly generated from PDMS. hESC-derived neurons and oligodendrocytes are viable in the platform and can be successfully cultured in the platform for a long period. Microchannels of the platform were shown to restrict and guide the growth of both cells and biochemical measurements can be performed in the system.

Conclusions: According to our studies, the developed platform is suitable for the co-culture of neurons and oligodendrocytes and has potential for various applications related to neuron-oligodendrocyte research.

T08-35B

Müller cells heterogeneity in vitro

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Müller cells constitute the predominant glial cell type in the retina. These cells display relevant functions in retinal homeostasis involved in structural support, metabolism, phagocytosis of neuronal debris, uptake of K^+, release of certain transmitters and trophic factors. Müller cells span the entire length of the retina and interact with virtually all cells types, but each Müller cell may only have to meet the requirements of its immediate neighbour neurons.

An emerging field of research in glial biology is the identification of phenotypic heterogeneity within glial cell types as Müller cells. The quest for cellular heterogeneity is important to know whether all Müller cells have the ability to perform all the functions or if there are different functional subpopulations scattered in the retina specialized in a different function each. Besides, it would be

interesting to know whether all Müller cells perform the same functions at once or if they take turns to maintain the homeostasis. If all Müller cells respond to damage from the same way, or some cells are more sensitive to damage than others is also not fully understood.

The aim of this study was to identify different phenotypic types of Müller cells in primary cells cultures using different cell markers.

In primary Müller cell cultures from adult rats or pigs, heterogeneity in marker expression, as glutamine synthetase (GS) or GFAP, has been observed. Müller cells expressing high amounts of GS or GFAP lie close to other cells that do not express or express a low concentration of these markers. Under the same experimental conditions, subpopulations of Müller cells express high amounts of only GS, only GFAP, both of them or low concentrations of both markers. However, depending on the conditions in which Müller cells are cultured, the expression patterns of these markers could be modified.

In addition to Müller cells heterogeneity, we have observed that Retinal Ganglion Cells (RGCs) prefer to grow on Müller cells that overexpress GS, when they grow on a confluent monolayer of Müller cells in co-culture. This fact may be due to this subpopulation of reactive Müller cells upregulate GS, which has been suggested to be a neuroprotective mechanism since it may limit glutamate neurotoxicity.

In conclusion, there is heterogeneity in Müller cells subpopulations *in vitro* attending to at least the GS and GFAP expression that should be deeply studied.

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T08-36B

Electrophysiological characterization of the prefrontal cortex and hippocampus connection in a genetic model of astrocytic dysfunction

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Astrocytes are a glial cell type predominantly present in the nervous system and are becoming recognized for their unique morphologic and phenotypic features that allow them to monitor their neighbour activity and dynamically respond to changes with intracellular calcium signalling. The deletion of IP3 receptor type 2 in mice was shown to block calcium oscillations specifically in astrocytes, impairing the function of the tripartite synapse. In order to assess the implications of astrocyte calcium signalling to the function of brain networks, behaviour and in vivo electrophysiological assessments were performed. Our goal was to evaluate the output response of neurons by analysing local field activity (local filed potentials, LFPs) from IP3R2KO mice and compare with activity recorded from their respective littermate wild-types. Specifically, LFPs were recorded from the hippocampus (HIP) and prefrontal cortex (PFC), critical regions for the computation of cognitive function. Regional activity as well as coherence analysis between both regions was performed. Morris Water Maze test was used to assess the behavior performance of the HIP-PFC link. Interesting correlations between behaviour performance and electrophysiological recordings were found, indication that theta activity synchronization seems to be implicated in MWM Reversal Learning task for IP3R2KO mice. This observation suggests a crucial role for astrocytic calcium in complex computation within the neuroglial-networks.

T08-37B

The pros and cons of studying astrocytic Ca^{2+} dynamics with genetically-encoded Ca^{2+} indicators: a high-resolution two-photon comparative analysis with synthetic dyes

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Astrocytes display a wide range of Ca^{2+} transients in response to synaptic activity that may reciprocally induce neuromodulation. Classically, astrocytic events were found to last tens of seconds and recruit large areas of the astrocyte. Recently, there has also been reported the existence of faster (sub-sec to few sec) Ca^{2+} activity in astrocytic processes. The fastest component of such activity involves sub-sec "focal" Ca^{2+} transients observed in small process sub-regions (reviewed in Volterra et al., Nat Rev Neurosci 2014), caused by synaptic release and blocked by interfering with GPRC or IP3 signaling in the astrocytes (Di Castro et al. Nat Neurosci 2011).

Notably, the fast Ca^{2+} activity in astrocytic processes was visualized using small, pipette-loaded organic calcium dyes such as Fluo-4 that have high signal fidelity, but also some drawbacks that in principle can be addressed by using genetically-encoded Ca^{2+} indicators (GECIs). However, groups that expressed GECIs in astrocytes via viral infection have so far reported only longer-lasting Ca^{2+} transients (duration ~5-10s), suggesting that GECIs may not yet equal the sensitivity of the small dyes for the fastest events. To test this, we systematically compared acutely loaded dyes (Fluo-4-AM and Rhod2-AM), and a genetically encoded cytosolic GCaMP3 in unperturbed hippocampal astrocytes of adult mice.

Using multiphoton imaging in the fast line-scan mode, we compared Fluo-4 and GCaMP3 in the same type of process segments up to 20 um from the soma. Also, because Rhod2 and GCaMP3 are spectrally separated, we simultaneously recorded signals from both dyes co-loaded into the same process.

We saw that Fluo-4 AM, Rhod2-AM and GCaMP3 can faithfully report the presence of longer (few sec) Ca^{2+} transients at a frequency matching that previously reported for "expanded" events recorded with pipette-loaded Fluo-4 (Di Castro et al. Nat Neurosci 2011). For this sub-group of events, GCaMP3 shows a superb SNR matching the quality of pipette-loaded dyes, and a greater dynamic range as compared to Rhod-2 AM. However, GCaMP3 tended to under-report the faster (sub-sec), smaller Ca^{2+} transients even compared to the AM-organic dyes.

In summary, the GCaMP3 generation of GECI is already able to report good part of the Ca^{2+} events in astrocytes with high fidelity. As for the fastest events, it is likely that the future GECI generations will parallel the quality and utility of Fluo-4, the current standard.

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T08-38B

Release of glutamate and ATP induced by optogenetic activation of astrocytes

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Question: Astrocytes are endowed with the capability of releasing transmitters (gliotransmitters) among which glutamate, D-serine, ATP, GABA and glycine have been identified. However, the mechanisms and conditions leading to the release of these different gliotransmitters are still highly debated.

Methods: We therefore aimed at using optogenetic activation of astrocytes to understand better gliotransmission and its consequence on neuronal activity.

Results: We crossed Cx30-cre-ERT2 (kindly provided by Frank W Pfrieger) and Floxed-ChR2-EYFP mice (Ai32; Jackson lab) to induce the selective expression of ChR2 in astrocytes. We first confirmed by immunocytochemistry that EYFP was specifically expressed in the vast majority of hippocampal and cortical astrocytes 21 days after an i.p. injection of tamoxifen. We also used acute hippocampal slices to characterize the membrane currents induced in astrocytes by blue light stimulations of variable duration. At a holding potential of -90 mV, light stimulation induced a fast inward current that peaked in few milliseconds (ms). If light was maintained, this initial peak was followed by a plateau of

lower amplitude and, after tens of ms, by a slowly increasing inward current that could last several seconds. We then recorded CA1 pyramidal cells to analyze the neuronal consequences of light-induced activation of ChR2 in astrocytes. We did not observed changes in neuronal membrane currents for short duration, full field, blue light stimulations. Yet, light pulses of more than 1 second reliably induced a sequence of depolarizing-hyperpolarizing responses that were not blocked by TTX (1 μM). The hyperpolarizing current was abolished by the adenosine A1 receptor antagonist DCPCX (200 nM), suggesting a release of ATP or adenosine by astrocytes. The depolarizing component was fully blocked by antagonists of NMDARs (50 μM D-AP5, 40 μM MK-801, 50 μM 7Cl-KYN) but not by the AMPA-KAR antagonist NBQX (10-20 μM). This NMDAR-mediated current was potentiated by the blocker of glutamate transporters TBOA (100 μM) but not affected by application of glycine or D-serine, which in our conditions failed also to induce any change in the baseline membrane current.

Conclusions: These results suggest that activation of ChR2 in astrocytes also induces the release of glutamate that targets specifically NMDARs expressed by CA1 pyramidal cells.

T08-39B

Activity-dependent neuroglial remodeling enhances extrasynaptic glutamate signaling and optimizes adaptive neuronal responses to a physiological challenge

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In addition to its classical synaptic actions, glutamate evokes a persistent excitation by activating extrasynaptic NMDA receptors (eNMDARs). By regulating extrasynaptic glutamate concentrations, astrocytes can modulate the degree of eNMDAR activation. Thus, the topography of the local neuroglial microenvironment is a factor expected to influence the strength of eNMDAR signaling. The magnocellular neurosecretory system in the supraoptic (SON) and paraventricular hypothalamic nuclei, plays a key role in bodily homeostasis. This system is characterized by a compact neuroglial microenvironment, in which astroglial processes tightly enwrap SON neurons. Remarkably, activity-dependent neuroglial remodeling, including retraction of astroglial processes, contributes to adaptive SON neuronal responses to a physiological challenge, such as dehydration. Thus, the magnocellular system constitutes an ideal model to study eNMDAR signaling in conditions where neurons are either tightly or loosely surrounded by astrocytic processes. We previously showed that ambient glutamate of a non-synaptic source, and whose levels are tightly controlled by the astrocyte glutamate transporter GLT1, activates a persistent excitatory current mediated by eNMDARs, stimulating SON neuronal firing activity. We found that activation of eNMDARs inhibited the magnitude of the transient A-type K^+ current I_A ($P< 0.01$), strengthening the SON neuronal input/output function. The eNMDAR-I_A coupling was Ca^{2+}-and PKC-dependent. Pharmacological blockade of GLT1 (DHK 300 μM) increased the basal degree of eNMDAR activation ($P< 0.01$), inhibited I_A magnitude ($P< 0.05$), and increased SON repetitive firing ($P< 0.001$). In addition, when rats were subjected to a dehydration challenge (48hs water deprivation), we found retraction of astrocyte glial processes, a basally diminished I_A magnitude ($P< 0.05$ vs. euhydrated (EU) rats) and a higher basal SON firing activity ($P< 0.01$ vs. EU). Finally, we found a blunted effect of GLT1 blockade on eNMDAR-mediated tonic current and SON firing activity ($P < 0.01$ *vs.* EU rats in both cases). Taken together, our studies support that (1) astrocyte modulation of eNMDAR signaling contributes to regulation of SON neuronal activity; (2) that this neuroglial interaction involves a functional negative coupling between eNMDARs and I_A; and (3) that activity-dependent neuroglial remodeling strengthened eNMDAR excitatory signaling, contributing in turn to neuronal homeostatic responses during a physiological challenge.

T08-40B

A novel open source tool to study astrocytic morphology

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Cell morphology analysis has proven to be a key tool to identify several cellular features: as shape, morphological adaptations and interactions with surroundings cells, giving a better understanding of its functions and role within the network.

Despite of the emerging importance of astrocytes in brain function, especially in synaptic transmission and plasticity, morphological alterations have been poorly studied. Assessing the morphology of astrocytic processes, their close structural association with synapses and their morphologic changes / remodelling will lead to a better understanding of the neuro-glia networks with putative functional implications.

Several remarkable softwares as Neurolucida, FilamentTracer by Imaris, Amira, Neuron 3DMA among others had been used to trace neurons and perform morphometric analysis. All of them have in common an associated license cost.

Here we present an open source software, Simple Neurite Tracer, to trace GFAP positive astrocytes labelled by immunofluorescence, in images collected by confocal laser scanning microscope from different conditions of rodent samples.

Simple neurite tracer is an open source plugin from ImageJ that performs morphometric analysis of dendrites by quantifying their number, ramifications, total length and tortuosity. It also provides detailed information about the complexity of the astrocytic morphology by sholl analysis.

We found this tool to be practical, reliable and most importantly to present less degree of user-dependent errors since it traces the cell on a semi-automatically manner. Therefore this software might be a valuable tool to assess astrocyte morphology in an easier and economic way.

T08-41B

Microglia in the early development of inhibitory cortical circuits

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Recent studies have revealed that microglia, the resident macrophages of the brain, contribute to the emergence and maturation of neural circuits during the early wiring of the mouse central nervous system. In particular, microglia have been shown to modulate the laminar positioning of *lhx6*-expressing interneurons during the embryonic and early-postnatal development of the somatosensory cortex. Indeed, absence, maternal immune inflammation (MIA) or genetic functional perturbation of microglia during embryogenesis affects *lhx6*-interneurons distribution in the somatosensory cortex, with subsequent postnatal long-lasting effects. However, the precise role of microglia in interneuron positioning and integration in somatosensory cortical circuits still remains to be investigated. Here, using a combination of experimental approaches in models of embryonic depletion, as well as MIA, we will present novel findings on the roles and mechanisms of microglia in the development of inhibitory circuits in the somatosensory neocortex. Since early defects in cortical networks formation are associated with the etiology of several neuropsychiatric disorders, our studies will contribute to a better understanding of microglia roles during normal and pathological brain wiring.

T08-42B

Implication of microglial fractalkine receptor in hypothalamic control of metabolism

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There is an emerging role of glial cells in sensing and mediating of various homeostatic functions. Our aim was to reveal the role of hypothalamic microglia in the regulation of energy homeostasis focusing on the state of negative energy balance (insulin-induced hypoglycemia).

C57Bl6/J mice were fasted overnight and sacrificed 1 hour after intraperitoneal insulin injection (1 IU/kg) in the next morning. Non-fasted and fasted control animals received vehicle injection. Quantitative and qualitative morphometrical analysis of Iba1 stained serial hypothalamic sections of insulin-injected animals revealed significant activation of microglia in the medial basal hypothalamus, the area that has been implicated in glucose and insulin sensing and regulation of food intake and energy expenditure.

Using c-Fos based functional anatomical mapping strategy we have identified neurons in the hypothalamic arcuate region that became activated in response to insulin-induced hypoglycemia. Histological analysis of double stained material (c-Fos and Iba1) at light microscopic level found these activated neurons to be surrounded by activated microglia after insulin challenge.

To explore the role of neuron-to-microglia communication in mediation of insulin-induced hypoglycemia, mice that are deficient in fractalkine signaling ($CX3CR1^{gfp/gfp}$) were injected with insulin or vehicle after fasting. Fractalkine receptor deficient mice did not develop severe hypoglycemia following insulin as did wild type (C57Bl6 background) littermates. By comparing insulin-induced c-Fos immunoreactivity in these mice, we found special subregions within the hypothalamic arcuate and median eminince region that became distinctly activated in response to insulin injection. Under resting conditions, the number and activation profile of hypothalamic microglia was not different in wild-type (C57Bl6/J) and $CX3CR1^{gfp/gfp}$ animals. However, in response to insulin-induced hypoglycemia, there was a transition shift of microglia from resting to activated morphological phenotype especially in the rostral and middle parts of the arcuate region and at the level of median eminence in wild type animals. Furthermore, these activational changes in the rostral arcuate nucleus were much less expressed in mice with impaired fractalkine signaling than in the controls.

Our results highlight the role of microglia in general-, and fractalkine signaling in particular, mediating hypoglycemic challenges to the hypothalamic neuronal circuit that regulates energy homeostasis.

T08-43B

$CD11c^+$ microglia are potent producers of IGF-1 during postnatal neurodevelopment

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Microglia are CNS resident immune cells that are known to be activated in response to pathological events. The role of microglia in neuroinflammation has been extensively investigated, and there is a growing interest in the function of microglia in the steady state and neurodevelopment. Microglia are defined as expressing a lower level of CD45 than blood-derived leukocytes, expressing fractalkine receptor CX3CR1 and lacking CCR2 chemokine receptor. In many neuroinflammatory and degenerative conditions a population of $CD11c^+$ microglia increase in proportion and number. In EAE they were shown to be effective antigen presenting cells for T cell proliferation, but they were a poor source of pro-inflammatory cytokines. We now show that they are a major source of neuroprotective insulin-like growth factor 1 (IGF-1) which suggests a protective rather than proinflammatory role for $CD11c^+$ microglia. In this study we show that numbers and proportion of $CD11c^+$ microglia greatly expand during postnatal development (PN3-PN5) and then decrease dramatically to less than 3% of total microglia as mice age to adulthood. In developing (PN4) brains $Iba1^+$ $CX3CR1^+$ $CD11c^+$ microglia are localized mainly in cerebellum and corpus callosum but also in cerebral cortex. $CD11c^+$ microglia sorted from neonatal (PN3-PN5) CNS expressed the great majority of IGF-1 mRNA compared to sorted CD11c negative microglia, astrocytes, neurons and OPCs. We thus identify $CD11c^+$ microglia as a potent and inducible source of IGF-1 necessary for neurodevelopment, neuroprotection and repair in the CNS

Poster topic 09
Ischemia and hypoxia

T09-01A

Sonic hedgehog controls NG2 glia differentiation following focal cerebral ischemia

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NG2 glia constitute a fourth glial cell type in the adult mammalian central nervous system that is distinct from other neural cell types. Several studies have shown that these cells display wide differentiation potential under pathological conditions *in vivo*, where they also give rise to a certain subpopulation of reactive astrocytes. Here we aimed to identify the role of Sonic hedgehog (Shh) in NG2 glia differentiation following focal cerebral ischemia. We used transgenic Cspg4-cre/Esr1/ROSA26Sortm14(CAG-tdTomato) mice, in which tamoxifen administration activates the expression of red fluorescent protein (tomato) specifically in NG2 glia. We employed the patch-clamp technique and immunohistochemical analyses to determine the differentiation potential of tomato-positive (tomato$^+$) NG2 cells *in vitro* as well as *in vivo* in non-injured or post-ischemic brains in the presence of drugs, which activate or inhibit Sonic hedgehog (Shh) signaling pathway. The focal cerebral ischemia (FCI) was induced by middle cerebral artery occlusion. Our *in vitro* study showed that cultured Tomato$^+$ cells isolated from non-injured brain display membrane and immunohistochemical properties corresponding to several cell phenotypes. They comprised NG2 glia (45.0 ± 3.9%), astrocytes (22.0 ± 4.1%), oligodendrocytes (18.7 ± 5.0%) and pericytes (13.3 ± 3.8%). The presence of Shh increased the number of tomato$^+$ astrocytes to 43.2 ± 7.8%, which indicates a shift in NG2 glia differentiation towards astrocytes. An outcome very similar to the influence of Shh was observed in the culture of NG2 glia isolated from the post-ischemic cortex displaying a significantly increased number of astrocytes (74.1 ± 5.4%) at the expense of tomato$^+$ NG2 glia. The hypothesis that Shh plays a role in NG2 glia differentiation was confirm by using an inhibitor of Shh signaling pathway, cyclopamine, the addition of which blocked the differentiation of NG2 glia into astrocytes in the presence of Shh as well as in cultures isolated from the ischemic cortex. We also targeted Shh signaling *in vivo* following ischemic injury. Blocking Sonic hedgehog signaling by cyclopamine *in vivo* decreased number of NG2 cells differentiating into astrocytes and conversely, activation of Sonic hedgehog signaling by SAG increased the number of NG2 cells differentiating into astrocytes after ischemia. Taken together, our data indicate that Shh is a key factor that controls the differentiation of NG2 glia after focal ischemia controlling the composition of gliotic scar in the vicinity of ischemic lession.

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T09-02A

Docosahexanoic acid confers neuroprotection in perinatal hypoxia-ischemia in rats

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One of the most common causes of mortality and morbidity in children is perinatal hypoxia-ischemia, so new and more effective neuroprotective strategies are urgently required, in order to minimize as much as possible the neurological consequences of this encephalopathy. In this sense, interest has grown in docosahexanoic acid (DHA), which is a long-chain omega-3 fatty acid, commonly found in fish such as salmon and tuna. The aim of the present work was to evaluate the possible neuroprotective effect of DHA when administered before hypoxic-ischemic brain injury in neonatal rats, determining infact area, oligodendroglial injury and long-term behavioral consequences.

P7 rats were randomly assigned to: one control, hypoxia-ischemia (HI) and HI animals that received a single dose of 1 mg/kg of DHA. Injury was induced by permanent ligature of the left common carotid

artery and then by asphyxia for 135 minutes with 8% O2. P14 brains were stained with Nissl and immunolabelled with MBP and on P90 we evaluated the long-lasting behavioral alterations.

The quantitative analysis of the infarct area showed a severe percentage of tissue loss in HI group in comparison with Control and DHA. Microscopic photographs and the semi-quantitative neuropathological scoring system demonstrated a damage located at the level of hippocampus and parietal cortex of ipsilateral hemisphere in HI group, damage was reducted by DHA. MBP-immunostaining pattern showed a significant decrease in the HI group in comparision with the Control, that was restored with DHA. On PN90, animals pretreated with DHA performed better at the T-maze, hole-board and novel object recognition tests than HI animals.

Our results suggest that a pretreatment with docosahexanoic acid led to a neuroprotective effect by reducting the infarct volume, ameliorating cell damage, preserving myelin and improving cognitive impairments.

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T09-03A

Mitochondrial dysfunction and aggravated oxidative stress mediate increased vulnerability of aging white matter to ischemia

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The risk for stroke increases with age and brain white matter (WM) inherently becomes more susceptible to injury as a function of age. WM structure and function are integrated; therefore age-related changes in the molecular architecture of WM determine the principal injury mechanisms and functional outcome. Axon function in aging WM recovers less due to increased excitotoxicity and bioenergetic failure. In this study, using 3-dimensional electron microscopy imaging we characterized the age-dependent modifications of axonal structures in mouse optic nerve, a pure WM tract, specifically focusing on mitochondria and endoplasmic reticulum (ER) to define the rapid exhaustion of energy capacity that underlies the increased vulnerability of aging WM to ischemia.

Mitochondria exhibit regulated dynamics to efficiently buffer Ca^{2+}, to produce sufficient ATP, and to effectively scavenge reactive oxygen species (ROS). Aging enhances mitochondrial fusion due to a mismatch in the expression.of mitochondrial shaping proteins and leads to mitochondrial aggregation because of reduced expression of Miro-1 (mitochondrial Rho GTPase) levels, a Ca sensor that stops mitochondrial movement when Ca levels rise. Interestingly, mitochondrial location with respect to the endoplasmic reticulum (ER) also shows notable changes with aging. These two organelles traffic in coordinated manner and maintain regions of close contact with each other to control lipid biosyhthesis, mitochondrial division and Ca signaling. The ER network not only considerably diminishes with aging but also the proportion of ER near mitochondria and near axon membrane decreases while the highest proportion of ER is found in axoplasm unassociated with mitochondria. Calnexin and calreticulin are components of ER resident quality control systems that promote correct protein folding and targets misfolded proteins for degradation. Their levels increase in young WM after ischemia whereas their baseline levels are considerably lower in aging WM and ischemia fails to upregulate them. Furthermore, aging WM generates significant amounts of oxidative injury by-products such as 3-NT and 4-HNE compared to young WM.

We propose that these age-specific alterations in WM cause rapid exhaustion of mitochondrial capacity and disruption of ER-mitochondrial interactions to meet the energy demand of axons leading to increased oxidative stress and render the tissue more vulnerable to an ischemic attack.

T09-04A

Neonatal hypoxic ischemic brain damages: early neuroprotective effect of lactate

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Introduction: The role of lactate in brain energetic metabolism has been challenged during the last 3 decades. If lactate was mainly considered as a waste product in the past, lactate is now regarded as a supplementary fuel[1] and, more recently, as a signalling molecule[2,3]. Indeed, recently, a new brain lactate receptor (GPR81) was discovered[2]. Previous works on a mice model of transient focal cerebral ischemia have shown that lactate can be neuroprotective[4]. This effect was also suggested in humans, in traumatic brain injury[5,6]. The aim of this study was to investigate the neuroprotective effect of lactate in a rat neonatal model of hypoxia-ischemia.

Methods: Neonate Wistar rat (were used in the study. The hypoxic-ischemic (HI) damage was obtained as described previously by Vannucci[7]. Pups received an intra-peritoneal injection of lactate before hypoxia or after HI insult. Control rats received an injection of NaCl 0.9% after HI, such as sham rats. Three hours after carotid occlusion, brain lesions were assessed by magnetic resonance diffusion weighted imaging at 4.7T. Volume of brain lesion, apparent diffusion coefficient (ADC) and fractional anisotropy (FA) were measured for each animal.

Results and Discussion: In a rat model of neonatal HI, intra-peritoneal injection of lactate allows a significant decrease (-33%) of the lesion sizes. Lactate was even more neuroprotective when injected after HI insult, highlighting its potential clinical interest. This effect appears early, as soon as 30min after lactate injection. Higher ADC values observed in HI-L rats suggest a decrease in the cytotoxic oedema quickly after lactate administration. These preliminary results reveal a neuroprotective effect of lactate in a hypoxia-ischemia context on neonate rats.

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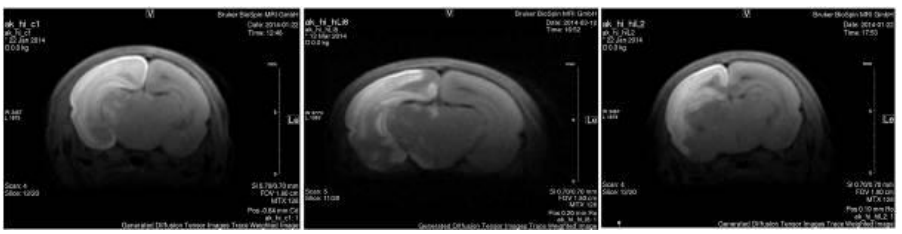
Image



T09-05A

Phagocytic astrocytes after brain ischemia

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Rapid clearance of neuronal debris, including neuronal cell corpses as well as axonal, dendritic and synaptic debris, is thought to be essential for the maintenance of brain functions and microenvironment. Such events include phagocytosis, which is thought to be limited to the "professional phagocytes", i.e., microglia in the brain. However, recent studies revealed that astrocytes have several phagocytosis-related molecules, and in fact, immature astrocytes participate in synapse elimination in the developing mouse brain, suggesting that astrocytes could be phagocytic cells as well. However, astrocytes have received only limited attention as phagocytes simply because they little show phagocytosis in the normal adult brain. Astrocytes are highly responsive to changes in brain environments including various brain injuries and diseases and dramatically change their characteristic features, and thus, we might have underestimated astrocytic phagocytosis. In this study, we show that a subset of reactive astrocytes become phagocytic after transient ischemic injury in the limited spatiotemporal pattern. After transient middle cerebral artery occlusion (MCAO), microglia were initially activated and transformed into highly phagocytic mainly in the ischemic core region, which was followed by activation of astrocytes in the penumbra region. Interestingly, such reactive astrocytes showed phagocytic phenotypes. Molecular-based characterization of these reactive astrocytes revealed that ABCA1 (*ced-7*) and its signaling cascade molecules, MEGF10 (*ced-1*), GULP1 (*ced-6*), both of which are highly implicated in engulfment, were increased. Using pharmacological and molecular biological techniques in cultured astrocytes, we identified that these molecules were responsible for astrocytic phagocytosis. Taken together, we demonstrated that astrocytes become phagocytic after MCAO with different spatiotemporal patterns of microglia. These findings suggest that astrocytes as phagocytes in the adult brain, should have distinct roles in regulation of brain functions.

T09-06A

Antioxidant treatments recover the auditory evoked potentials alteration and reduce morphological damage in the inferior colliculus after perinatal asphyxia in rat

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Perinatal hypoxic-ischemic (HI) encephalopathy remains one of the main causes of disabilities in term-born infants such ashearing impairments. The auditory brainstem response (ABR) has been shown to be very sensitive to low blood oxygen concentrations, which may damage the neurosensory cells of the Organ of Corti or loss of brainstem neurons; such as those of the inferior colliculus (IC).One of the key events in HI pathogenesis is the early generation of reactive oxygen species (ROS) and consequently, supplementation or treatment with antioxidants has been proposed to be an appropriate target area for novel therapies.

The aim of the present work was to evaluate morphofunctionally the effect of a panel of antioxidants on HI-induced auditory deficits. To this end, we studied the effects of nicotine, melatonin, resveratrol andDocosahexaenoic acid(DHA) on the neonatal auditory system via measurement of auditory evoked potentials and characterization of the morphological integrity of the IC.

7-day Sprague-Dawley rats were randomly assigned into six experimental group: **Control,** pups with neither ischemic nor hypoxic injury, **HI** group, animals with permanent left carotid occlusion and reduction of O_2 to 8%, **HI+D**, animals treated with DHA (1mg/kg), **HI + N**, animals treated with nicotine (1,2mg/kg), **HI+M**, HI animals treated with melatonin (15mg/kg) and **HI+RV**, animals treated with resveratrol (20mg/kg), and ABR were measured, animals were sacrificed and the inferior colliculi isolated, paraformaldehyde fixed and stained for morphological study of neurons, astrocytes and white matter.

Latencies of the waves IV and V were significant longer in the HI group respect to the control group. However, all groups treated with antioxidant agents showed similar wave latencies to those of the control group. Staining of brain sections microscopically revealed signs of early neuronal damage, astrocytes reactivity and reduce in myelin basic protein (MBP) induced by the hypoxic-ischemic event, whereas sections of non-ischemic control animals or treated animals did not.

Taken as a whole, the present prospective study presents for the first time a correlation between the functional and morphological aspects underlying the antioxidant induced amelioration of HI induced brainstem damage. Thus, antioxidant treatments were found to provide effective neuroprotection to the immature auditory system before a perinatal hypoxic-ischemic event.

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T09-09A

Characterization of the polarization state of microglia and infiltrating peripheral macrophages in a transient Middle Cerebral Artery Occlusion model in mice

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Microglia and macrophages from the peripheral circulation contribute importantly to the inflammatory response after ischemic stroke. There is evidence that microglia and macrophages can shift between pro-inflammatory M1 cytotoxic to anti-inflammatory M2 pro-repair states. Recent studies have examined macrophage/microglia polarization in brain ischemia, however these studies have not distinguished between the polarization state of microglia and infiltrating macrophages. We have used the LysM-EGFP knockin mouse in which peripheral macrophages and neutrophils are tagged with EGFP to distinguish them from microglia at the site of cerebral ischemia and studied the expression of M1 polarization markers after a transient Middle Cerebral Artery Occlusion (tMCAO). The middle cerebral artery was occluded in LysM-EGFP mice with a 9-0 suture and reperfusion was allowed by removing the suture 90 minutes later. Animals were trans-cardially perfused and tissue taken at 24h, 72h and 7 days post-tMCAO for immunofluorescence staining. There were very few infiltrating EGFP+ cells in the penumbra or core of the infarct at 24h. Their numbers increased markedly at 72h after reperfusion and comprise of both infiltrating macrophages and neutrophils; and are distributed evenly within the lesion core. At 7 days the EGFP+ cells which at this time point consist mainly of infiltrating macrophages appear to be aggregated and clustered in the core of the infarct. We also did double immunofluorescence labeling for two well established M1 markers (CD86 and CD16/32) combined with Iba-1 (a microglia/macrophage marker). Our preliminary results show that both CD86 and CD16/32 are mainly expressed in $Iba1^{+}$ /$EGFP^{-}$ (microglia) but not in $Iba1^{+}$ /$EGFP^{+}$ (macrophages) cells. Both markers were found in the border and penumbra regions of the lesion. These results show that resident microglia become activated and polarized to a predominantly M1 state while infiltrating macrophages, which are mainly localized in the core of the lesion do not appear to be M1 polarized, and thus could be in a less pro-inflammatory state. Additional work is underway to assess the expression of M2 markers. The use of the LysM-EGFP mice have allowed us to distinguish the polarization state of microglia and infiltrating peripheral macrophages at the site of cerebral ischemia.

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T09-10A

Glutamate release mechanisms in pre-myelinated CNS white matter

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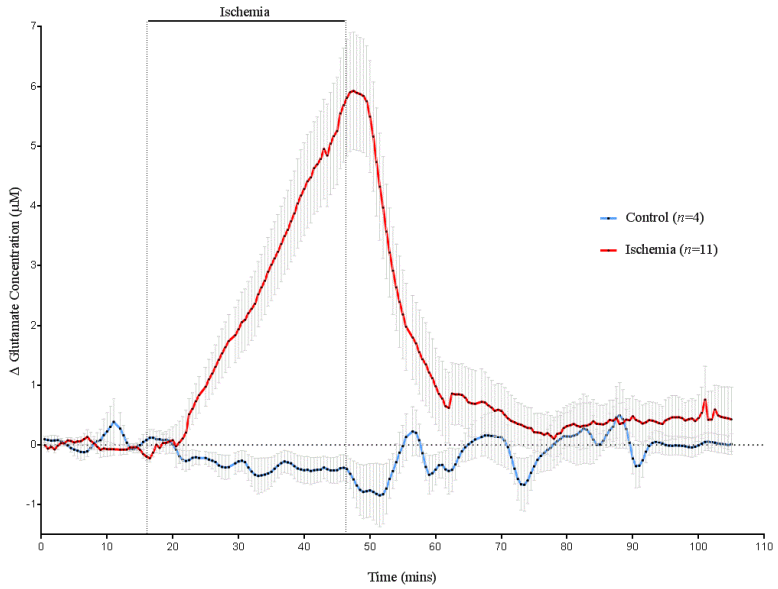
Background & Objective: Ischemic injury to developing white matter (WM) can lead to a selective pattern of injury known as *periventricular white matter injury*, the most common pathological substrate associated with cerebral palsy. There is evidence that the over-activation of ionotropic glutamate receptors mediates the ischemic cell injury/death of both developing oligodendrocytes and small pre-myelinated axons which populate WM regions at this age. Here we investigate possible mechanisms of ischemia-induced glutamate release from developing WM.

Methods: Using glutamate-specific microbiosensors, real-time extracellular glutamate concentrations were recorded from inside the postnatal day 10 (P10) rat optic nerve. Anoxia was found to affect glutamate recordings using this approach, so the metabolic inhibitor, rotenone, combined with glucose deprivation, was employed as a model of chemical ischemia. Extracellular glutamate was recorded during 30 minutes of modelled ischemia under a variety of conditions and pharmacological treatments, aimed at blocking potential release mechanisms. Compound action potential (CAP) recordings were used as a measure of functional injury/recovery.

Results: The mean resting extracellular glutamate concentration was 2.5±0.59μM (mean±SEM). Glutamate concentrations increased steadily during modelled ischemia, increasing by 6.22±0.99μM before returning to baseline following reperfusion. CAP amplitude was irreversibly reduced to 10.43±3.38% following reperfusion, reflecting a large degree of functional injury associated with the rise in extracellular glutamate. Loss of nerve excitability during ischemia initiated at a similar time as the start of the glutamate rise, but evolved more rapidly.

Conclusion: Ischemia evokes a robust release of intracellular glutamate in developing WM, leading to a significant increase in extracellular glutamate concentrations.

Image



T09-01B

Astrocyte diversity in response to stroke

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Astrocytes respond to injury by undergoing a phenotypic change referred to as reactive astrocytosis. It has long been recognized that reactive astrocytosis is not an all-or-none phenomenon but rather a gradated process with differing levels of severity. However, the levels of reactive astrocytosis are not well defined, nor is it clear exactly how astrocytic functions are changing as a result of these alterations or how these changes affect neural repair and recovery from injury. Using both cortical and white matter models of ischemic stroke, we have sought to define the morphologic, phenotypic, and transcriptomic changes astrocytes undergo as a function of their distance from the site of injury.

One clear way in which astrocytes respond to injury is via morphologic change, yet with traditional staining methods it is not possible to visualize the full extent of alteration in the elaborate astrocytic arbor. Therefore, we have developed a system to deliver limiting dilutions of astrocytic-specific lentiviruses driving new molecular reporters that allow clearer visualization. Using these reporters in combination with 3D reconstruction, we have quantified differences in astrocytic morphology by distance from infarct in both fibrous and protoplasmic astrocytes. This analysis identified distinct zones of astrocyte morphology after injury. An analysis of the relative expression of different functionally relevant astrocytic markers across these morphologically distinct zones suggests that these morphologic changes are accompanied by phenotypic changes, reinforcing the concept that these represent distinct astrocyte populations.

In order to get a more complete view of the ways in which these different astrocytic populations change after stroke, we have developed a way to specifically isolate ribosomally loaded astrocyte RNA from discrete astrocytic zones. By crossing RiboTag mice, in which a ribosomal subunit expresses a hemagglutinin tag in a Cre-dependent fashion, with a GFAP-Cre line, we have generated animals in which tagged ribosomes are specifically expressed in astrocytes. Using laser capture microdissection, immunoprecipitation, and RNAseq, we can then generate ribosomally associated transcriptomic profiles of different astrocytic zones after stroke. These data sets can be analyzed for both overall changes in specific genes as well as profiles of differential alternative splicing between zones. Together, these data provide detailed evidence of diverse astrocytic populations after stroke.

T09-02B

Impact of an ischemic episode on the physiology of Bergmann glial cells

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Cerebral ischemia is characterized by partial or total interruption of the blood supply to the brain resulting in glucose and oxygen deprivation to brain cells. The series of cellular processes that are unleashed by cerebral ischemia are complex. The severe reduction in oxygen and glucose induces decreases in ATP production and dramatic changes in extracellular K^+ concentration, pH of intracellular and extracellular space and lactate production. The disruption of energy metabolism in the ischemic tissue rapidly lead to membrane depolarisation and neurotransmitters are released into the extracellular space. In the cerebellum, the impact of an ischemic stress has been extensively studied in Purkinje cells, the only neuronal output of the cerebellar cortex. It has been shown that glutamate released from overexcited fibers and from reversal of glutamate transporters, is the principal cause of the dramatic, anoxic depolarization in Purkinje cells. However a detailed understanding of the astrocytic response to cerebellar ischemia and the potential influence of astrocyte to ischemia outcome is still lacking.

Bergmann glia (BG) are radial gial cells that form networks of electrically coupled cells underling complex anatomical and functional interactions with the neurons of the cerebellar cortex. Using an in vitro model of cerebral ischemia, the oxygen and glucose deprivation (OGD), several basic features of astrocytic reaction to ischemia are analyzed. Patch clamp and calcium imaging experiments performed in cerebellar slices from adult mice revealed that BG respond to OGD with a progressive membrane depolarisation that is paralleled with a sustained cytosolic calcium increase. Double patch-clamp recordings between Purkinje neurons and BG reveal different responses to OGD in these cell types. Furthermore, we measured extracellular potassium concentration changes during OGD by using ion-sensitive microelectrodes. Our results indicate an important correlation between the BG membrane depolarisation and the extracellular K^+ dynamics during OGD.

T09-03B

The immune receptor Mincle in microglia is a key initiator of tissue damage in ischemic stroke

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Mincle is a pattern recognition receptor thought to play a role in innate immune responses to sterile inflammation, but its contribution to ischemic injury is not well understood. We show that mice lacking Mincle had significantly improved infarct size and functional outcomes from experimental transient focal ischemia. Concordantly, they experienced lower infiltration of monocytes and neutrophils into the brain which in turn produced less inflammatory cytokines. Mincle expression was restricted to microglia and infiltrating cells in brain and periphery, and was not detectable in mouse or human neurons, astrocytes or endothelial cells. Unlike previously studied pattern recognition receptors, bone marrow chimeras demonstrated that the presence of Mincle in the central nervous system, not in peripheral myeloid cells, was the critical regulator of poor outcome under ischemia/reperfusion injury. In agreement, genetic deletion of Mincle in mouse microglia altered the molecular response of microglia to ischemic conditions. These findings implicate Mincle in the initiation of microglial responses to ischemic injury.

T09-04B

Brain energy metabolism is impaired by the propagation of focal ischemic damage

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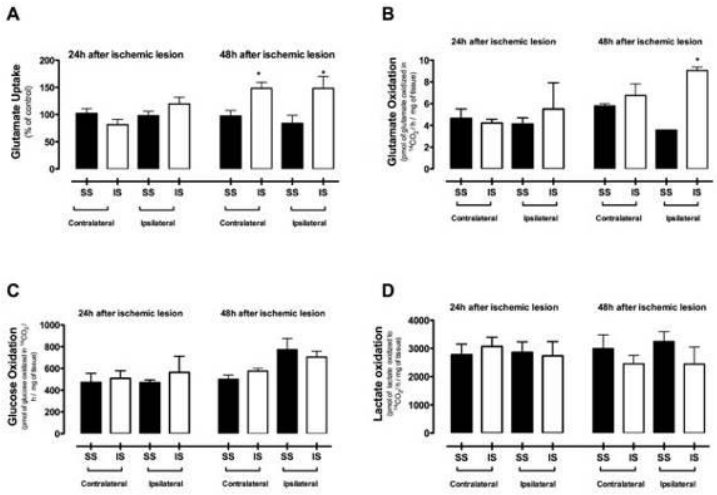
Acute ischemic stroke begins with severe focal hypoperfusion, preventing ATP synthesis and initiating a series of neurochemical processes known as ischemic cascade, producing cellular death. These processes alter cerebral substrates availability and utilization. Cerebral injury continues over hours and even days, expanding the infarcted core. Considering the roles of astrocytic metabolism in excitotoxicity and energy production, this study evaluated the effect of injury propagation on the energy metabolism 24h and 48h after focal permanent ischemia (FPI). The FPI was induced surgically by thermocoagulation of the blood in the pial vessels of the motor and sensorimotor cortices in adult (90 days old) male Wistar rats. Animals were divided in two (2) groups: sham (S) and ischemia (I). We performed the cylinder test to access the asymmetry score for each animal. For the I group, only animals with a score between 70 to 90% were used for the biochemical experiments. Brain infarct volume was measured using the 2,3,5-tetraphenyltetrazolium chloride (TTC) staining method. The biochemical analyses were performed in both ipsilateral and contralateral brain hemispheres, using tissue around the lesion focus (penumbral zone). Glutamate uptake was performed in slices using ^{3}H-glutamate and oxidation of three different substrates (glutamate, glucose and lactate) was measured by the CO_2 production marked with C^{14} by in a scintillation counter and expressed as cpm.mg^{-1}. The FPI model by thermocoagulation produces a markedly dysfunction of the impaired forelimb 24h and 48h after surgery, as observed in the cylinder test, followed by an average infarct volume of 119,188 mm^3 (n=5). No dysfunction of the forelimb neither infarct volume were observed in the S group. We noticed a significant increase in glutamate uptake in both hemispheres 48h after FPI (Fig.1A, $P<0.05$). Glutamate oxidation is also increased at the same time point, but only in the ipsilateral side, indicating more utilization of this substrate for energy production (Fig.1B, $P<0.05$). The FPI didn't affect glucose utilization (Fig.1C); however, after 48h of FPI, the animals showed a decreased in lactate utilization in both hemispheres (Fig. 1D, $P<0.05$). Considering the results above, the FPI model showed to modulate energy metabolism and lactate utilization, but didn't affected glucose metabolism. Further analysis involving glutamate and lactate transporters expression must be realized to clarify the pathways related in ischemic brain damage.

Image



T09-05B

A new *in vitro* model of focal ischemia: towards the understanding of re-oxygenation specific damage in the white matter

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The optic nerve (ON) is a good model for the study of pathological alterations in the white matter (WM) during ischemia. Similarly to the ON, other models successfully reproduce *in vitro* the effect of oxygen-glucose deprivation (OGD) in the WM. However, a model able to isolate the damage exclusively produced during re-oxygenation of the WM is unknown. Here ONs from rats and mice were used in order to develop a model to better understand this phenomenon. ONs were kept in aCSF containing (in mM): NaCl, 126; KCl, 3; NaH2PO4, 2; MgSO4, 2; CaCl2, 2; NaHCO3, 26; glucose, 10; pH, 7.45, bubbled with 5% CO2 / 95% O2 and maintained at 37°C. For OGD, aCSF was replaced by glucose-free aCSF saturated with a 95% N2/5% CO2 mixture. The chamber atmosphere was switched to 5% CO2 / 95% N2 during OGD perfusion. Compound action potentials (CAPs) were evoked and recorded with glass electrodes and CAP peak-to-peak amplitude or area were used to assess changes in excitability. Non-recoverable CAP loss from the ON indicates irreversible failure of axon function.

In young rats (P10-P24), CAP failed after 1h of OGD (2.5% remaining) and returned to 19.6 and 17.9% of control values when measured in the 1st or 2nd hour after re-oxygenation at 37ºC. When applied at 34ºC, OGD produced the same drop in CAP (2.5% CAP left), but afforded a recovery of 72.3 and 90.8 % when measured at the 1st or 2nd hour after re-oxygenation at 34ºC, respectively. When OGD was kept at 34ºC, but re-oxygenation was maintained at 37ºC, the recovery was still higher than OGD at 37ºC, 50.1% and 44.7% after 1 or 2 h or re-oxygenation. When the ON was locally damaged by 37ºC OGD, and the rest of the ON protected at 34ºC (during OGD only), a recovery of 49.9% was initially observed in the 37ºC re-oxygenation, but CAP dropped to 11.4% after 2h. In adult rats, we observed a similar pattern of protection by 34ºC during OGD only, with recoveries of 66.0 and 67.5% in the 1st and 2nd hours of re-oxygenation at 37ºC. However, using a local 37ºC OGD (with 2.1% remaining CAP area) did not produced a secondary damage like in young rats ONs, and the recovery was 55.9 and 57.9% in the 1st and 2nd hour at 37ºC. In adult mice ONs, in contrast, CAP dropped to 9.3% when a local OGD 37ºC was applied, and recovered to 40.5% after the 1st h, but dropped again to 15.6% in the 2nd h of re-oxygenation.

These results here suggest a mixed 34/37ºC OGD followed by a 37ºC re-oxygenation in the ON of young rats and adult mice as a new model of WM secondary damage induced by re-oxygenation after ischemia. The model offers new possibilities of understanding the cell death mechanism pertaining to this period.

T09-06B

Differential effects of intranasal epidermal growth factor treatment on the subventricular zone and dentate gyrus after chronic perinatal hypoxia

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There are no effective treatments available that improve function in the growing population of very preterm infants (<32 weeks gestation) with neonatal brain injury. Diffuse white matter and gray matter injury are common long-term findings in these children and contributes to their chronic neurodevelopmental and cognitive impairment. Using sublethal chronic perinatal hypoxia (Hyp; postnatal day P3-11) as a clinically relevant mouse model of very preterm brain injury, we have recently demonstrated that administration of intranasal epidermal growth factor (HB-EGF; P11-14) stimulates a response of endogenous EGF receptor (EGFR)-expressing progenitor cells in the white matter and promotes cellular, biochemical and functional recovery. It is established that injury results in a significant expansion of the neural stem cells (NSCs) and progenitor cells that reside in the subventricular (SVZ) and the hippocampal dentate gyrus subgranular zone (SGZ). However, it is unknown whether administration of intranasal HB-EGF treatment after Hyp: i) provides an additive stimulatory effect on newly generated NSCs and progenitor cells in these regions; and ii) results in a differential response between the SVZ and SGZ. We demonstrate that, in both the SVZ and SGZ, Hyp induces a significant increase in phosphorylated-EGFR and EGFR-ligand expression immediately after Hyp, but not at later time points. In the SVZ, HB-EGF treatment after Hyp resulted in: i) a significant expansion of Sox2- and Ascl1-expressing progenitor cells; ii) a significant, but transient expansion of NG2-expressing oligodendrocyte progenitor cells (OPCs); and iii) prevention of Hyp-induced expansion of Pax6-expressing cells and doublecortin (DCx)-BrdU-positive cells. Conversely, in the SGZ, HB-EGF treatment had an additive effect on both DCx-BrdU-positive cells and newly generated neuronal NeuN-positive cells. To determine the importance of EGFR signaling during development and recovery from Hyp injury, ongoing studies are being conducted to remove EGFR with an inducible Cre-system. HB-EGF treatment has differential effects in the SVZ and SGZ, in particular on neuroblast formation. Understanding the benefits of this differential effect on functional outcome after developmental brain injury is crucial in order to develop intranasal HB-EGF treatment as a potential therapeutic strategy for neonatal brain injury caused by very premature birth.

T09-07B

Protein profiling in penumbra after local photothrombotic infarction in the rat cerebral cortex

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Stroke-induced irreversible cell damage in the brain infarction core leads to lesion propagation to adjacent tissues and to formation of transitional zone, penumbra. We studied the changes in morphology and protein expression profile in the penumbra 1 h after focal tissue infarction induced by the local photodynamic treatment (i.v. administration of the cell-impermeable photosensitizer Bengal Rose, diode laser 532 nm, beam diameter 3 mm) in the rat cerebral cortex. Decrease in the laser light intensity and longer exposure provided expanded penumbra (up to 1.5 mm width). Morphological studies included standard histological staining and transmission electron microscopy. Microarrays “Panorama Antibody Microarray - Neurobiology” (Sigma-Aldrich) against 224 neuronal and signaling proteins were used for proteomic study. At the optical and ultrastructural levels, edema, vacuolization and destruction of organelles were observed in neurons, glial cells and capillaries in PTI core, adjacent and peripheral (much less) penumbra regions. Up-regulation of proteins involved in maintaining neurite integrity and guidance (NAV3, MAP1, CRMP2), myelinization and neuroglial interactions (PMP22); intercellular interactions (N-cadherin); synaptic transmission (glutamate decarboxylase, tryptophan hydroxylase, MUNC-18-3, and synphilin-1); mitochondria quality control and mitophagy (PINK1 and Parkin); ubiquitin-mediated proteolysis and tissue clearance (UCHL1, PINK1, Parkin, synphylin-1); and signaling proteins (PKBα, ERK5) in penumbra could be associated with tissue recovery. Downregulation of PKC, PKCβ1/2, and TDP-43 could also reduce tissue injury. Therefore,

despite significant morphological alterations in penumbra, the protein expression changes were directed mainly to protection and tissue recovery. Some upregulated proteins can potentially serve as markers of protection processes in penumbra. Supported by grants of RFBR (14-04-00741), RSF (14-15-00068) and Russian Ministry of Education and Science (Research organization #790).

T09-08B

Antihypoxic properties of Glial cell line-derived neurotrophic factor (GDNF) in the acute normobaric hypoxia *in vitro*

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Astrocytes are considered as active participants in the functioning of neural networks, able to regulate various aspects of neuronal metabolism and modulate the synaptic plasticity under the influence of stress-factors such as hypoxia. Neuron-astrocyte communicationis regulated by a variety of systems, one of the most active is the GDNF. GDNF can activate different intracellular processes stimulating the cell survival and their differentiation, neurite outgrowth and gene expression of neuronal regeneration. The aim of the investigation was to study the influence of GDNF on the functional neuronal network activity and on the viability of cells in the hypoxia model *in vitro*.

Studies were performed using hippocampal cells dissociated from 18-days embryonic CBA mice. Hypoxia model was performed after 14 th day *in vitro* by replacing the normoxic culture medium by a medium with low oxygen for 10 minutes. For the detection of patterns of spontaneous calcium oscillations we used fluorescent calcium dye Oregon Green 488 BAPTA-1 AM and a confocal microscopy. The viability of cells was evaluated according to the percentage ratio between the number of dead cells stained by propidium iodide. For the immunostaining principal cytoskeleton protein of neurons (MAP2) and astrocytes (GFAP) were marked in dissociated hippocampal cultures.

The carried out experiments revealed that hypoxia to cause irreversible changes in the spontaneous calcium activity and increase the number of dead cells by day 7 after hypoxia. There was a significant (ANOVA, $p<0,05$) decrease in the number of cells, exhibiting the calcium activity and the calcium oscillations frequency as well as the increase of the duration of calcium oscillations. Moreover, hypoxia leads to destructive changes in the morphological structures of neurons and astrocytes in dissociated hippocampal cultures. The neuronal outgrowth reduction and the modifications in the form of astrocytes were found 7 day after hypoxia. Astrocytes were combined into the single morphologically inseparable conglomerates. Preventive application of GDNF (1 ng/ml) neutralizes the negative hypoxic effects. The number of living cells as well as the number of cells, exhibiting the functional calcium activity, were significant (ANOVA, $p<0,05$) higher than in the control cultures. Thus, we suppose that GDNF has strong antihypoxic properties.

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T09-09B

Chronic stress exacerbates neuronal loss associated with secondary neurodegeneration and suppresses microglial-like cells following focal motor cortex ischemia in the mouse

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Post-stroke patients describe suffering from persistent and unremitting levels of distress. Using an experimental model of focal cortical ischemia in adult male C57BL/6 mice, we examined whether exposure to chronic stress could modify the development of secondary thalamic neurodegeneration (STND), which is commonly reported to be associated with impaired functional recovery. We were particularly focused on the modulatory role of microglia-like cells, as several clinical studies have linked microglial activation to the development of STND. One month following the induction of cortical ischemia we identified that numbers of microglial-like cells, as well as putative markers of microglial structural reorganisation (Iba-1), complement processing (CD11b), phagocytosis (CD68), and antigen presentation (MHC-II) were all significantly elevated in response to occlusion. We further identified that these changes co-occurred with a decrease in the numbers of mature neurons within the thalamus. Occluded animals that were also exposed to chronic stress exhibited significantly lower levels of Iba-1 positive cells and a reduced expression of Iba-1 and CD11b compared to the 'occlusion-alone' group. Interestingly, the dampened expression of microglial/monocyte markers observed in stressed animals was associated with significant additional loss of neurons. These findings indicate that the process of STND can be negatively modified, potentially in a microglial dependent manner, by exposure to chronic stress.

T09-10B

Rapid microglial actions contribute to excitotoxic responses and brain injury after cerebral ischemia

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Question: Inflammation plays a crucial role in the pathogenesis of cerebral ischemia. Inflammatory actions of microglia, the main inflammatory cells in the brain have been widely studied, but their functional contribution to brain injury is controversial.

Methods: Here we have established a novel, two-photon microscopy-based approach to study early microglial responses in a remote filament model of experimental stroke in mice. In this experimental model, occlusion of the middle cerebral artery and induction of reperfusion are tightly controlled, allowing the assessment of vascular, neuronal and microglial responses in the brain at a millisecond time scale for several hours *in vivo*. By using genetically encoded calcium indicators and transgenic microglia reporter mice, we have assessed changes in neuronal network activity, blood brain barrier injury, Ca^{2+} levels in the tissue and microglia activation simultaneously, in real time.

Results: We show that excitotoxic neuronal injury in the ipsilateral cerebral cortex is delayed by several hours after the onset of ischemia. Microglia contact injured neurons in an activity dependent manner. Microglia also react rapidly to early signs of vascular injury, preceding the breakdown of the blood brain barrier and isolate cells showing signs of oxidative stress in the injured brain. Selective depletion of microglia prior to experimental stroke results in altered vascular and excitotoxic responses and lack of spreading depolarisations, leading to markedly increased infarct size.

Conclusions: Collectively, our investigations link early microglial inflammatory responses to brain injury and in spite of some known neurotoxic actions mediated by microglia, our data suggest that these cells could protect the brain from excitotoxic and vascular injury after cerebral ischemia. Understanding the cellular and molecular mechanisms through which microglia contribute to stroke outcome could have important implications to the treatment of stroke and other forms of brain injury.

Poster topic 10
Myelin

T10-01A

The TAM receptor Tyro3 acts as a promyelinating factor on oligodendrocytes

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Question: The TAM family of receptor-tyrosine kinases, comprising Tyro3, Axl and Mertk, are widely expressed in the central nervous system (CNS). While Axl and Mertk have been characterised in their role as regulators of processes such as phagocytosis and inflammation little is known about the function of Tyro3. Mice deficient in the TAM ligand Gas6 show more severe demyelination and a delayed recovery in the cuprizone model of demyelination. Whilst Gas6, Axl and Mertk gene expression levels are increased during cuprizone-induced demyelination, Tyro3 levels are significantly decreased in a manner coincident with the loss of the myelin component, myelin basic protein (MBP). Furthermore, it was previously demonstrated that Gas6 directly increases myelination both *in vitro* and *in vivo*. As Tyro3 is more abundantly expressed in the CNS than the other TAMs and is upregulated in mature oligodendrocytes we hypothesised that the positive effects of Gas6 on myelination are mediated through this receptor. **Methods**: To test this hypothesis two cohorts of wild-type (WT) and Tyro3$^{-/-}$ mice (3-4/group) were subjected to three weeks of cuprizone-induced demyelination. Immunohistochemical and electron microscopical analyses were used to examine oligodendroglial and microglial cell numbers as well as numbers of myelinated axons in the corpus callosum (CC). We also used co-cultures of dorsal root ganglion neurons from WT Sprague Dawley rats and oligodendrocytes from C57Bl/6 WT and Tyro3$^{-/-}$ mice to assess the role of Tyro3 in regulating myelination *in vitro*. **Results**: Whilst we did not observe any changes in the number of myelinated axons in the CC of Tyro3$^{-/-}$ as compared to WT mice post cuprizone treatment these animals had significantly more small unmyelinated axons in the rostral region of the CC. Surprisingly, we found this change in both cuprizone-challenged as well as unchallenged Tyro3$^{-/-}$ mice. Our co-cultures using oligodendrocytes from Tyro3$^{-/-}$ mice revealed a reduced number of MBP^{+} myelin segments as compared with WT counterparts. **Conclusions**: These results suggest that Tyro3 positively regulates myelination through oligodendrocytes adding further evidence to a diversification of TAM receptor functions. Further investigations need to be done to elucidate the role of Tyro3 in the regulation of calibres of unmyelinated axons.

T10-02A

Membrane glycoprotein M6B is a novel component of the Node of Ranvier

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Question: Myelin serves to insulate axons to ensure efficient propagation of action potentials from one Node of Ranvier to the next. The myelin sheath is formed by Schwann cells and oligodendrocytes in the peripheral nervous system and central nervous system, respectively. The interaction between the axon and the myelin is mediated by proteins that are arranged on either side of the glial-axonal interface in organized domains centered around the Node of Ranvier. In the cellular membranes on either side of the nodal gap are found cell adhesion molecules (CAMs). Intracellularly, scaffolding proteins connect these CAMs to the cytoskeleton, and extracellularly, the CAMs interact with each other across the gap. Moreover, within the nodal gap several specific ECM components have been identified. Ultimately, the nodal components serve to restrain ion channels within the node to allow efficient saltatory conduction. Considering the importance of proper node formation and maintenance, we suggest that many nodal components have yet to be identified. What may these yet unidentified components of the node be?

Methods: For expression analysis, we performed RT-PCR of various tissues and cells. For *in vitro* localization analysis, we prepared myelinated dorsal root ganglia (DRG) cultures from E13.5 WT and M6B knockout mice. For examination of localization *in vivo*, we have used immunohistochemistry of teased Sciatic Nerve and Sciatic Nerve section from rat, WT mice and M6B knockout mice.

Results: We have discovered that the proteolipid family member Membrane Glycoprotein M6B resides at the Node of Ranvier. *In vitro*, we found M6B at the node in myelinating DRG cultures. Using cultures prepared from M6B knockout mice, we found that both nodes and their development precursors appear normal. Furthermore, we found M6B at the node in mouse and rat sciatic nerve. M6B is expressed in Schwann cells and seems to be localized to the glial side of the node. In sciatic nerves isolated from M6B KO mice, the nodal gap remains and contains known nodal proteins.

Conclusion: Here we report the discovery of Membrane Glycoprotein M6B as a novel nodal component. The nodes of M6B KO mice appear normal suggesting that other proteins might compensate for the lack of M6B in these animals.

T10-03A

Myelin-Associated Glycoprotein (*MAG)* mutation causes Pelizaeus Merzbacher disease-like disorder

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Myelinating oligodendrocytes and Schwann cells are involved in mass production of membrane proteins, and are therefore particularly susceptible to disruption of the secretory pathway. Defects in the secretory pathway of myelinating cells can lead to a broad range of pathologies such as Pelizaeus-Merzbacher Disease (PMD) and multiple sclerosis (MS). Myelin-associated glycoprotein (MAG), also referred to siglec-4, has been subject to extensive research due its role in axonal outgrowth and myelination, though its physiological function remains unclear. To date, no natural occurring mutations in human MAG have been documented. Here we report a novel single nucleotide mutation at serine 133 of MAG, substituting serine for arginine leading to Pelizaeus Merzbacher disease (PMD) like diseases in humans. By utilizing diverse genetic and biochemical approaches In a variety of cell types, including Schwann cells and oligodendrocytes, we are able to demonstrate that the S133R mutation has a destructive effect on MAG folding and posttranslational processing, resulting in S133RMAG endoplasmic reticulum (ER) retention and subsequent endoplasmic reticulum associated degradation (ERAD). Together, these data suggests that substitution of serine for arginine in postion 133 of MAG leads to ERAD mediated loss of function, which might be the mechanism underlying the pathologies exhibited in patients. Moreover, these findings may allow future development of therapeutic strategies for patients baring this mutation.

T10-04A

mTORC1 regulation of Schwann cell myelination

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Neuregulin-1 type III has emerged as a crucial axonal cue driving the onset of myelination and the radial growth of myelin in Schwann cells. However, the relative importance of the downstream pathways in these two processes is still a matter of debate. We recently showed that disruption of mTOR complex 1 (mTORC1) signaling results in a severe hypomyelination. Nevertheless, a formal

proof that increasing mTORC1 would be sufficient to phenocopy the hypermyelination due to neuregulin-1 type III overexpression is still lacking. To our surprise, we show here that a simple increase in mTORC1 during development does not lead to hypermyelination, but rather blocks Schwann cells at the promyelinating stage. We will present a model that reconciles these tantalizing findings.

T10-05A

Upregulation of early differentiation factors (Id2, Sox2) in neuropathic nerve: Pathogenetic or Protective?

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Myelin Protein Zero is the most abundant structural protein in myelin of the PNS. In humans, more than 120 mutations in P0 are associated with hereditary neuropathies. Deletion of serine 63 causes Charcot-Marie-Tooth (CMT) 1B disease in humans and a similar demyelinating neuropathy in mice. P0S63del protein is misfolded and is retained in the ER where it gives rise to a dose-dependent Unfolded Protein Response (UPR). The UPR results in the activation of transcriptional and translation control programs that reduce protein synthesis and increase the folding and degradative capacity of the cell. Usually, when this first response is not sufficient the cells may activate apoptosis resulting in cell death. However, in P0S63del Schwann cells there is no cell death suggesting that these cells may respond differently to chronic stress. Transcriptomic analysis performed on P28 P0S63del nerves showed increased expression of transcription factors normally present only in the early phases of differentiation such as c-Jun, Sox2 and Id2. Sox2 and Id2 are involved in the differentiation of central nervous system neurons and glia but their function in the PNS is not completely understood. We show that overexpression of Sox2 in dorsal root ganglia (DRG) cultures strongly reduces myelination whereas Id2 overexpression does not appear to have the same effect. However, Id2 null DRGs show an increase in myelinated fibers, suggesting a negative role of this protein in Schwann cell differentiation. To verify if Sox2 and Id2 expression in neuropathic nerves could contribute to the hypomyelination observed in CMT1B mice, we generated P0S63del/P0Cre/Sox2 floxed and P0S63del/Id2 null mice . Surprisingly, our results indicate that ablation of Sox2 or Id2 (or both) in CMT1B background severely worsen the phenotype. This suggests that the overexpression of early differentiation factors in Schwann cell under chronic ER-stress is a protective mechanism.

T10-06A

The influence of mDomino/p400 on Schwann cell development in mice

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The ATPase mDomino/p400 is a subunit of the Tip60/p400 chromatin remodeling complex known to be involved in differentiation and senescence as well as in regulation of gene expression and DNA damage response in various cell types. In myeloid cells, the ATPase interacts with the transcription factor MZF-2A during growth, differentiation and tumorigenesis. During embryonic hematopoiesis, mDomino/p400 coordinates the expression of the Hox gene cluster, while it influences expression of cell cycle regulatory genes in embryonic fibroblasts with important consequences for cell proliferation. One of its major roles is the exchange of the H2A histone variant with H2A.Z in promoter regions. The chromatin remodeling activity of mDomino/p400 is additionally involved in DNA damage response and cellular senescence. Up to now mDomino/p400 has not been studied in glial cells and myelination. By analyzing the peripheral nervous system of mice in which mDomino/p400 was selectively deleted in immature Schwann cells by Cre-mediated recombination, we unravel an important role of this protein in Schwann cell differentiation. Postnatally animals develop signs of hypomyelination and peripheral neuropathy with a reduction of mature, myelinating Schwann cells and myelinated axons. mDomino/p400-deficient mice have a thinner and translucent nerve with less myelin. mDomino/p400 also interacts with the Sox10 transcription factor. Current studies are focused on the molecular

mechanisms of mDomino/p400 in Schwann cells and their potential link to the transcriptional Schwann cell regulator Sox10.

T10-07A

Microglia-derived extracellular vesicles regulate the proliferation and differentiation of oligodendrocyte precursor cells

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Microglia have an enormous plasticity in their responses to CNS injury and can also display beneficial functions, playing an important role in fine-tuning inflammatory responses and promoting tissue repair (Miron E et al., 2013 Nat Neurosci). Despite the important role of microglia in various neurodegenerative disorders, the mode(s) of action of these cells in fostering or inhibiting CNS repair are far from being elucidated. Novel insights are needed in order to prevent the deleterious effects of inflammatory microglia and modulate them into a neurosupportive phenotype.

Here, we investigated through what interactions and signals primary microglial cells orchestrate the endogenous reparative responses mediated by Oligodendrocyte Precursor Cells (OPCs), the glial cell type able to generate mature, myelinating oligodendrocytes (Fumagalli M et al., 2011 Front Biosci). We focused on extracellular vesicles (EVs) released from reactive microglia, which have been recently reported to play a crucial role in intercellular signalling between microglia and adjacent brain cells (i.e. neurons and astrocytes; Prada I et al., 2013 Glia; Verderio C et al., 2012 Ann Neurol). In detail, we explored whether EVs released from pro-inflammatory M1 or pro-regenerative M2 microglia can boost or block the proliferation and terminal differentiation of OPCs. Fluorescence microscopy analysis of OPCs exposed to EVs for 24h in the presence of the proliferative marker EdU showed that EVs produced by M1 cells inhibit OPC proliferation, while EVs released by M2 microglia increase OPC proliferation. Immunocytochemistry and western-blot analysis of markers of mature oligodendrocytes (e.g. CNPase and myelin basic protein MBP) revealed that 48h exposure to EVs derived from either M1 or M2 microglia, but not resting cells, promotes OPC maturation, with EVs derived from M2 cells displaying higher differentiation activity. Moreover, we also observed that a 10-day exposure to microglia-derived EVs favors myelin deposition in an *in vitro* system of OPCs co-cultured with DRG neurons. The possible role of microglia-derived EVs in modulating the OPC response is also suggested by preliminary data in an *in vivo* model of lysolecithin-induced corpus callosum demyelination.

Globally, these results unveil EVs as key players in microglia-OPCs cross-talk and suggest that the phenotype acquired by microglia greatly influences the proliferative/differentiation potential of OPCs.

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T10-08A

Dysfunctional NF-κB and brain myelin formation

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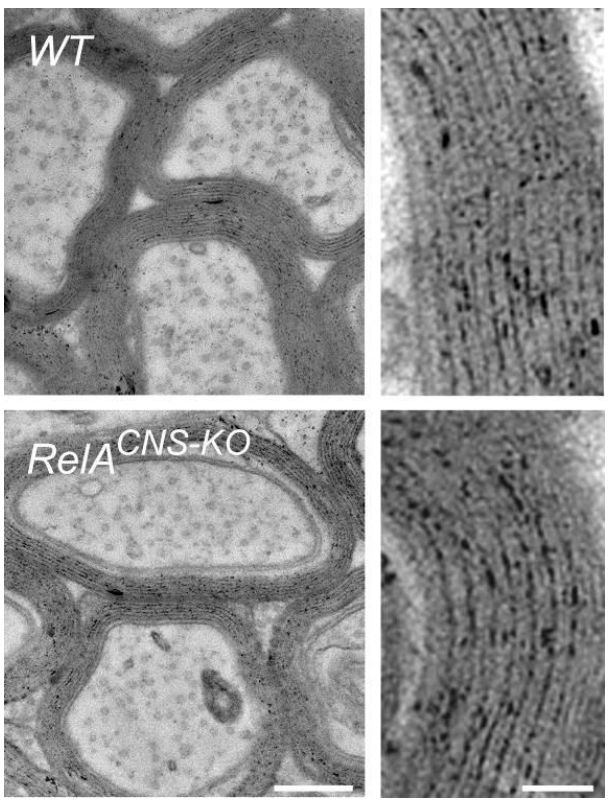
Activation of the transcription factor NF-κB in Schwann cells (SC) and CNS-intrinsic oligodendrocytes (ODC) has been supposed as a key regulator for myelin formation in the developing nervous system by transcriptional upregulation of myelin-associated proteins. In patients carrying a genetic duplication of the *Xq28* locus their overall dysfunctional NF-κB activation is associated with distorted brain myelination (Philippe *et al.*, 2013). Additionally, experimental demyelinating nerve injury activates NF-κB, and inhibition of NF-κB in denervated SC significantly influences axonal regeneration (Morton *et al.*, 2012). Here, we addressed the cell-type specific role of the transcriptional activator subunit of classical NF-κB, RelA (p65), for myelination and regeneration of axonal fibers in the CNS of mice. Using immunohistochemistry, we found significant expression of RelA in CAII-positive ODC of the naïve optic nerve (ON). Expression levels in ON and brain were almost completely abolished in transgenic mice with conditional deletion of RelA in neurons and macroglia including ODC ($RelA^{CNS-KO}$). Notably, there was no discernable impairment in structure or myelination of sensory axons in adult ON (Fig. 1), thus showing that CNS-intrinsic activation of classical NF-κB via RelA is not essential for proper CNS myelin formation *in vivo*. Functionally, tracer-based MR imaging (MEMRI) revealed normal active Mn^{2+} uptake into retinal ganglion cells (RGC) and transport along the retino-tectal projection, indicating unimpaired fiber vitality in the absence of RelA. Finally, functional parameters of visual acuity and contrast sensitivity were indistinguishable between wild-type and $RelA^{CNS-KO}$ mice. Following crush injury of the ON, we found activation of RelA in ODC within the lesion site. At four weeks post-injury, the level of myelin basic protein (MBP) was significantly reduced in the degenerating ON of $RelA^{CNS-KO}$ mice, as compared to wild-type specimens. To elucidate functional effects of reduced MBP expression by peri-lesional ODC, we investigated axonal regeneration of RGC into the lesioned ON in mice with oligodendrocyte-specific (CNP1-Cre) deletion of RelA ($RelA^{ODC-KO}$). We found a significant increase in fiber lenghts in $RelA^{ODC-KO}$, compared to wild-type mice, indicating that NF-κB regulates the expression of ODC-specific axonal growth inhibitors in the post-lesional CNS. Currently, we are studying myelinogenesis in RelA,c-Rel double knockout mice to explore functional compensation mechanisms under RelA deficiency by the NF-κB subunit c-Rel.

Figure 1: Ultrastructural micrographs showing normal myelination in the optic nerve of young adult $RelA^{CNS-KO}$ mice, as compared to wildy-type (*WT*) mice. Scale bar: 150 nm (left), 25 nm (right).

Image



T10-09A

Differential modulation of the juxtaparanodal complex in Multiple Sclerosis

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Myelinated fibers are divided in discrete subdomains around the Na_v-enriched nodes of Ranvier: the paranodes, where axoglial interactions occur, the juxtaparanodes, where voltage-gated potassium channels (VGKCs) are found in a complex with TAG-1 and Caspr2, and the internode. Perinodal changes have been reported in Multiple Sclerosis (MS) with functional consequences for the axon. Here we report on alterations of all three juxtaparanodal proteins TAG-1, Caspr2 and VGKCs in normal appearing white matter (NAWM), perilesion and chronic lesion areas in post-mortem white matter tissue from MS patients compared to control white matter. Using immunoblot and mRNA analysis as well as immunohistochemistry we found that the molecular organization and maintenance of juxtaparanodes is affected not only in lesions and perilesions but also in NAWM in chronic MS. The three molecules analyzed were differentially altered. TAG-1 clustering at juxtaparanodes was reduced in NAWM; TAG-1 and Caspr2 are diffused in perilesions and absent in lesion areas. VGKCs were the most susceptible of the three components as they were no longer enriched at juxtaparanodes either at the NAWM or the perilesion and demyelinated plaques. While the protein levels of the three molecules showed only a tendency for reduction in the plaques, there was a significant upregulation of Caspr2 mRNA in the lesions accompanied by a transcriptional increase of paranodal Caspr, indicating an axonal homeostatic mechanism. Our study is the first to comparatively analyze at the protein and molecular level the localization and expression of three components of the juxtaparanodal complex in chronic MS. Additionally, a phenotype of juxtaparanodal disruption is described for the first time in MS NAWM.

T10-10A

N-WASP-dependent molecular mechanisms involved in PNS myelination

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Question: In the peripheral nervous system (PNS) myelinating Schwann cells (SCs) sort larger axons, ensheath them and eventually elaborate myelin sheaths around axons by spirally wrapping their plasma membranes. During myelination SCs undergo extensive changes in cell shape, a process mediated through actin-regulating proteins, such as Neuronal Wiskott-Aldrich Syndrome protein (N-WASP).

N-WASP is characterized by several functional domains, including the N-terminal WH1 domain, followed by a basic region and a GTPase-binding domain, prolin-rich region and the C-terminal VCA region. N-WASP controls actin reorganization via interaction with different proteins resulting in several biological functions, such as filopodium formation, endocytosis and regulation of membrane curvature.

Previous work done in our laboratory indicates that N-WASP is essential for membrane wrapping, longitudinal extension and myelination by SCs, probably through regulation of actin cytoskeleton.

In the present study we ask what are the cellular and molecular mechanisms underlying the role of N-WASP in SCs differentiation and myelination.

Methods: To determine which function of N-WASP mediates myelination by SCs we employed retroviral delivery of full-length N-WASP or different mutant N-WASP proteins to N-WASP-deficient SCs in an in-vitro dorsal root ganglia (DRG) culture. Myelin rescue was assessed by immunostaining analysis.

Results: Our preliminary results indicate that expression of exogenous N-WASP by retroviral delivery is sufficient to rescue the non-myelinating phenotype of N-WASP null SCs. In addition, rescue experiments using mutant versions of the protein show that the WH1, CRIB, proline-rich and CA regions of N-WASP are essential for myelination. However, several myelin segments were detected in cultures expressing N-WASP lacking the WH1 domain. Interestingly, these few myelin segments were shorter than segments formed following expression of full-length N-WASP, indicating that longitudinal extension of the myelin unit is regulated by members of the WIP family that bind to the WH1 domain.

Conclusions: Overall, our findings suggest that N-WASP interacts with several effectors, which may cause rearrangement of the actin cytoskeleton required for elaboration of myelin sheathes by SCs.

Further in-vivo and in-vitro studies should help unravel how N-WASP-mediated actin dynamics controls the highly complicated process of myelin formation.

T10-11A

MicroRNA miR-145-5p represents a novel MS therapeutic target through its regulation of critical myelination regulator MYRF

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Progressive multiple sclerosis (MS) is a debilitating disease in which demyelinated lesions form in the central nervous system (CNS). In healthy individuals, demyelination leads to recruitment of CNS myelinating cells, oligodendrocytes (OLs), and subsequent remyelination. However in progressive MS, recruited OLs fail to remyelinate denuded axons, leading to neurodegeneration. One characteristic of MS lesions is abnormally high expression of microRNA miR-145-5p. Importantly, miR-145-5p is predicted to target myelin gene regulatory factor (*MYRF*), a transcription factor which activates expression of critical myelin genes such as myelin associated glycoprotein (*MAG*). In this study, we aimed to determine whether or not miR-145-5p does in fact directly target *MYRF*, and how altering normal expression of miR-145-5p affects OL maturation.

Stable cell lines overexpressing miR-145-5p were created using Oli-neus, immortalized oligodendrocyte progenitor cells also capable of becoming astrocytes under certain conditions. Differentiating cells overexpressing miR-145-5p showed significant downregulation of *MYRF*. Further, a severe reduction in MAG expression was observed. Dual luciferase assays confirmed direct targeting of *MYRF* by miR-145-5p at two binding sites. Differentiating cells also displayed aberrant morphology, more closely resembling astrocytes than OLs. However, they showed no loss in expression of OL markers nor gain in expression of astrocyte markers.

Taken together, these data show that reduced expression of *MYRF* and its downstream target MAG are due to direct targeting of *MYRF* by miR-145-5p. Further, while the overexpression of miR-145-5p results in severe alterations in morphology, cells maintain their OL identity. This research may be important in developing remyelination therapies for progressive MS.

T10-12A

Investigating the mechanistic basis of cholesterol-mediated myelination

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During development, oligodendrocytes synthesize large amounts of specialized myelin membrane to ensheath and insulate axons. Cholesterol, a major component of the myelin membrane, has a physiological role in myelin membrane formation because it influences membrane fluidity and associates with myelin proteins such as myelin proteolipid protein. Surprisingly, cholesterol also is required by oligodendrocytes to express myelin genes and wrap axons. How cholesterol mediates these distinct features of oligodendrocyte development is not known. One possibility is that cholesterol promotes myelination by forming membrane microdomains that facilitate signal transduction within the

cell. Signaling cascades influenced by membrane microdomains include the PI3K/Akt pathway, which acts upstream of mTOR, a major driver of myelination. Therefore, we hypothesized that cholesterol promotes myelin gene expression and axon ensheathment by facilitating mTOR signaling in oligodendrocytes. To test this hypothesis we manipulated cholesterol levels and PI3K/Akt/mTOR signaling in zebrafish and assessed myelin gene expression and axon wrapping. First, by performing immunohistochemistry to detect phosphorylated S6 ribosomal protein, an indicator of mTOR activity, we learned that oligodendrocytes of cholesterol-deficient zebrafish larvae have abnormally low levels of mTOR signaling. This is consistent with the idea that activation of the PI3K/Akt/mTOR requires cholesterol. Second, activating the PI3K/Akt/mTOR pathway, by interfering with the function of the pathway inhibitor PTEN, rescued myelin gene expression and axon wrapping in cholesterol-deficient larvae, indicating that the requirement for cholesterol in myelination is mediated by mTOR signaling. Because mTOR promotes synthesis of lipids, including cholesterol, our data raise the possibility that cholesterol synthesis and mTOR signaling engage in positive feedback to promote the formation of the large amounts of membrane necessary for myelination.

T10-13A

Investigation of myelin maintenance and turnover by inducible MBP knock-out in adult mice

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In the mouse central nervous system myelin is formed during development by early differentiated oligodendrocytes. Since myelin proteins are long lived, we ask the question how after the completion of myelination the myelin sheath is maintained in adulthood and turned over. Theoretically, this could be achieved by replacement of aged oligodendrocytes by newly differentiated OPCs, or by cell-based turnover of individual myelin components. To investigate this we generated mice with a floxed exon 4 of the MBP locus (exon 1 of the classical MBPs) and Cre-ERT2 under control of the PLP promotor. By tamoxifen-induction at the age of 8 weeks we knocked out MBP in differentiated oligodendrocytes. At around 5 months after induction the mice developed a phenotype characterized by hind limb tremor. At time points of 2, 4, 6 and 12 months after KO induction electron microscopic evaluation revealed structural changes in the myelin sheaths becoming visible at 4 months and widespread at 6 months after KO. These are characterized by *shiverer*-like non-compact membrane loops emanating from the myelin sheath at the inner tongue with progressive transformation of the myelin sheath into shiverer-like membrane processes in the vicinity of naked axons. Biochemical evaluation is in process. We conclude that the myelin sheath is continuously turned over with an almost complete exchange of MBP within 6 months.

T10-14A

Characterization of DUSP15/VHY as a regulatory target of Sox10 and Myrf

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Myelin formation by oligodendrocytes is required for proper signal transduction along axons. As a consequence, research focusing on the development of oligodendrocytes from oligodendrocyte precursor cells (OPCs) is of great interest for cell replacement therapies in myelin disorders.

The differentiation process of OPCs into mature oligodendrocytes is regulated by several transcription factors such as Sox10 or Myrf. Recently, it has been shown that Sox10 and Myrf cooperatively activate many terminal differentiation genes and induce myelination. Here, we show that DUSP15 is one of the genes upregulated during oligodendrocyte differentiation. Quantitative RT-PCR demonstrated a considerable upregulation of DUSP15 transcripts in differentiating oligodendrocytes. Western Blots and immunocytochemical stainings confirmed this upregulation in primary oligodendroglial cultures on the protein level. Additionally, we characterize the Dual Specificity Phosphatase DUSP15/VHY as one of the common target genes of Sox10 and Myrf in differentiating oligodendrocytes. We demonstrate by EMSA that both transcription factors bind to specific recognition sites in the DUSP15 promoter. In Luciferase assays, a synergistic activation of the DUSP15 promoter by Sox10 and Myrf was revealed. Collectively, our data identify DUSP15 as a new joint target of

Sox10 and Myrf during OPC differentiation. Future studies will elucidate the biological function of DUSP15 in the process of OPC differentiation.

T10-15A

Role of Jun activating binding protein 1 (Jab1) in Central Nervous System (CNS) myelination

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Oligodendrocytes (OLs) are the myelin forming cells of the central nervous system (CNS). Myelin allows rapid impulse propagation and contributes to preserve axonal integrity of myelinated axons. Demyelination and prolonged failure to remyelinate axons can result in axonal degeneration and neuronal cell death, which correlates with clinical disability of several neurodegenerative disorders including multiple sclerosis (MS).

Molecular pathways involved in myelin formation during development and upon regeneration are in part overlapping. Recent findings suggest that they may originate from extracellular matrix (ECM) signals and control several aspects of OL behavior including differentiation, proliferation and survival. We recently described Jun activating binding protein 1 (Jab1) as a nuclear molecule downstream to ECM-derived signals and involved in the regulation of Schwann cell differentiation, proliferation and survival. Loss of Jab1 in Schwann cells causes peripheral nerve demyelination and axonal degeneration.

Here we present preliminary data showing that Jab1 plays a role in OL behaviour and CNS degeneration. Conditional inactivation of Jab1 in OL, by CNP-Cre transgene, results in CNS hypomyelination, subsequent progressive demyelination and axonal degeneration and microglia infiltration. These features are evident in different CNS areas including optic nerve, corpus callosum and spinal cord. Surprisingly, mutant OL precursors normally differentiate into mature OLs, proliferate and survive. Postnatal inactivation of Jab1 by means of PLP-ERT2-Cre transgene does not affect myelin maintenance.

In conclusion Jab1 plays a role in CNS myelination and OL control of axonal integrity.

T10-16A

Analysing the role of Sox2 in regulating Schwann cell myelination during development and after injury

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Sox2 is a member of the SRY-related HMG box (SOX) family of transcription factors and is best known for its ability to regulate cell proliferation and pluripotency. In the peripheral nervous system (PNS), Sox2 is expressed in the nuclei of immature Schwann cells prior to myelination and is down-regulated as Schwann cells begin to myelinate. Using a Schwann cell specific P0-CRE line together with a Sox2-IRES-GFP transgenic mouse line that expresses Sox2 and GFP following recombination, we have fully tested the effects of maintaining Sox2 expression in Schwann cells during development in vivo. Our data shows that both at early post-natal time points and into adulthood, Sox2 triggers

Schwann cell proliferation and represses myelination, in addition to reducing nerve conduction velocity and both sensory and motor function in vivo.

Following nerve injury, Schwann cells have been shown to re-express Sox2, down-regulate myelin proteins and adopt a repair-supportive phenotype, to facilitate axonal regeneration and functional repair. Once nerve regeneration and repair is complete, Sox2 expression is again reduced and Schwann cells differentiate back into myelinating Schwann cells. We wanted to analyse the effects of maintaining Sox2 expression in Schwann cells following peripheral nerve injury. Using the same transgenic mouse line, we were able to show that maintained Sox2 expression following injury resulted in an increase in proliferation, reduced myelin protein expression and a reduction in functional recovery 21 days post sciatic nerve crush injury. These experiments demonstrate for the first time that Sox2 is a key negative regulator of Schwann cell myelination in vivo.

Key words: Schwann, peripheral nervous system, Sox2, Krox20, myelin.

T10-17A

***In vivo* pathogenesis of demyelination in an animal model of multiple sclerosis**

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Multiple sclerosis (MS) is an immune-mediated demyelinating disease of the human central nervous system (CNS). Although demyelination represents the major histopathological hallmark of the disease, currently the mechanisms that mediate myelin loss are not fully understood. Our study aims to understand how myelin is damaged in experimental autoimmune encephalomyelitis (EAE), the most commonly used animal model of MS, by combining two-photon *in vivo* imaging with confocal microscopy and correlated ultrastructural analysis.

For our experiments we used Biozzi ABH mice, known to initially develop a chronic relapsing-remitting form of EAE following immunization with myelin oligodendrocyte glycoprotein, which later evolves into a steady progression of the disease. Our findings suggest that oligodendrocyte damage in EAE follows an "outside-to-inside" pattern, affecting myelin first and only later leading to oligodendrocyte loss. *In vivo* imaging of fluorescently labeled axons and myelin sheaths revealed that in active demyelinating lesions, myelin is detached from axons in bulb-like structures that we call “myelinosomes”. We can detect such myelinosomes in acute and in chronic stages in the animal model, as well in biopsies from MS patients. Correlated light and electron microscopy confirmed the presence of myelinosomes in acute lesions and shows that myelinosomes can in some cases encompass the entire myelin sheath, while in others they consist of single or few myelin leaflets. We are currently investigating the molecular interactions that underlie the formation of such myelinosomes to gather further insight into the mechanisms that mediate immune-mediated demyelination.

T10-18A

Role of microglia in myelin turnover?

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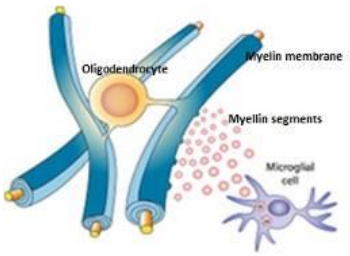
During central nervous system development oligodendrocytes synthesise large amounts of specific proteins and lipids to generate myelin. The synthesis, maintenance and turnover of such enormous amounts of myelin membranes come at a price for myelinating oligodendrocytes. Specifically, myelin turnover might be problematic for a cell that is able to form up to 80 different myelin internodes of tightly stacked membrane while harbours little cytoplasm and lysosomes, where degradation usually

occurs. How do molecules that are trapped within the numerous layers of tightly compacted membrane get access to the degradation system?

We have observed that cultured oligodendrocytes release exosomes/microvesicles into the extracellular space where they are taken up by microglia through a process defined as micropinocytosis. Therefore, we hypothesise that oligodendrocytes might outsource myelin membrane disposal to phagocytic cells such as microglia and macrophage. Another possibility of how myelin could be turned over is by stripping entire myelin segments by microglia. To test whether microglia/macrophages contribute to myelin turnover, we generated mice in which the lysosomal degradation pathway is specifically blocked in microglia due to deletion of the gene encoding Rab7. This protein is essential for the fusion of late endosomes to lysosomes. Therefore, in these mice a traffic jam is created within the endosomal-lysosomal system in microglia which allows us to detect internalized myelin fragment within the cell.

These mice develop pathology with signs of lysosomal storage associated with myelin-derived material in microglia. We propose that myelin turnover occurs partially by the shedding of myelin fragments into the extracellular space which can be taken up by microglia.

Image



T10-19A

Cystine/glutamate antiporter is essential for oligodendrocyte survival and its blockage exacerbates experimental autoimmune encephalomyelitis

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The cystine/glutamate antiporter (also known as system xc-) is a membrane-bound transport system responsible for the uptake of extracellular cystine and release of intracellular glutamate. It is the major source of cystine in most cells, and it is a main regulator of extracellular glutamate concentration in the Central Nervous System (CNS). Since cystine is the key amino acid in the biosynthesis of glutathione, and glutamate is the most abundant neurotransmitter, the cystine/glutamate exchanger is a central

player both in antioxidant defense and glutamatergic signaling, two events critical to brain function. However, the distribution of the cystine/glutamate antiporter in the CNS has not been well characterized. We have here analyzed the expression of the catalytic subunit of the cystine/glutamate antiporter, xCT, by immunohistochemistry in histological sections of the forebrain and spinal cord. We detected labeling in neurons, oligodendrocytes, microglia and oligodendrocyte precursor cells, but not in $GFAP^+$ astrocytes. We also analyzed xCT expression and function by qPCR and cystine uptake in primary cultures of the major cell types of the CNS, and we detected that neurons and oligodendrocytes have much higher levels of expression of the antiporter. Chronic blockage of the cystine/glutamate antiporter with specific inhibitors or external glutamate showed decreased viability mainly in cultured oligodendrocytes. Importantly, xCT-KO mice with chronic experimental autoimmune encephalomyelitis exhibited more severe motor symptoms and tissue damage (i.e., lower numbers of oligodendrocytes) in the spinal cord than wild type mice. Together, these results reveal that the cystine/glutamate antiporter is highly expressed in oligodendrocytes whereby it is key to the maintenance of cell homeostasis and plays a protective role in autoimmune-mediated demyelination. We suggest that pharmacological potentiation of xCT may ameliorate the outcome of multiple sclerosis and other demyelinating diseases.

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T10-20A

CNS Myelin sheath is stochastically built by homotypic fusion of myelin membranes within the bounds of an oligodendrocyte process

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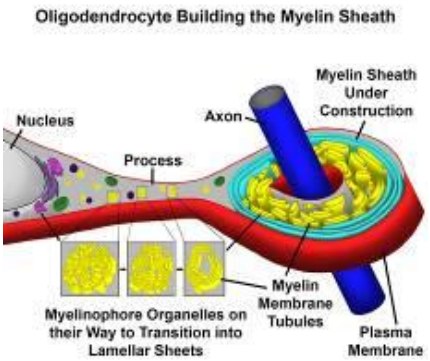
Myelin - the multilayer membrane that envelops axons - is a facilitator of rapid nerve conduction. Oligodendrocytes form CNS myelin; the prevailing hypothesis being that they do it by extending a chemically modified process that flattens out while circumnavigating the axon multiple times. The implication is that the myelin sheath is a slightly modified oligodendrocyte plasma membrane. In actual reality, the two membranes are compositionally distinct. It is pertinent to ask how the myelin sheath is built. To this end, we investigated myelin sheath formation in pure cultures of mature ovine OLGs and in embryonic (E) avian optic nerves at stages E12, E15, E17 and E21 by electron microscopy, immuno-electron microscopy and three-dimensional electron tomography. We report that myelin membranes exist as independent structural entities. They are synthesized, tubulated and packaged in organelles - named here myelinophore organelles - within the oligodendrocyte perikaryon. Myelin membranes are matured in and transported by myelinophore organelles within an oligodendrocyte process. On its approach to an axon, the OLG process splits to produce two branches that, after embracing the axon, overlap at the opposite end. The myelin sheath is generated by a stochastic fusion of myelin membranes into lamellar layers within the confines of an oligodendrocyte process. The assembly of the myelin sheath begins at the far end from the axon and advances towards it. These findings challenge the notion of myelin resulting from a wrapping motion of an oligodendrocyte process and open up new avenues in quest of understanding myelination in health and disease.

Image



T10-21A

Role of Ire1/Xbp-1 pathway in S63del neuropathy

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P0 glycoprotein is an abundant product of terminal differentiation in myelinating Schwann cells. The mutant P0S63del causes Charcot-Marie-Tooth (CMT) 1B disease in humans and a similar demyelinating neuropathy in transgenic mice. P0S63del protein is retained in the endoplasmic reticulum (ER) of Schwann cells where it elicits an adaptive unfolded protein response (UPR) featured by activation of PERK, ATF6 and Ire1/Xbp-1 pathways. We have previously reported that activation of CHOP and GADD34, two downstream mediators of PERK, is pathogenetic in S63del mice (Pennuto, 2008; D'Antonio, 2013). However the role of the other branches of the UPR remains to be investigated. In particular, activation of Ire1/Xbp-1 pathway, which has been shown to promote cell survival during prolonged ER stress, may be a critical adaptive response attenuating S63del toxicity. To modulate Xbp-1 pathway in S63del neuropathy, we generated a new mouse model with Schwann cells-specific ablation of Xbp-1 and in parallel we are exploiting S63del dorsal root ganglia (DRG) explant cultures in which Xbp-1 signaling is modulated by gain/loss of function approaches. Preliminary results show that inhibition of Xbp-1 splicing by 4μ8c (Cross, 2010) decreases myelination in S63del DRG cultures without significantly altering myelination in wild-type DRG cultures. These data suggest that activation of Xbp-1 signaling has a protective role in S63del neuropathy. The phenotype of wild-type and S63del mice lacking Xbp-1 specifically in Schwann cells is currently under investigation. Deciphering the role Ire1/Xbp1 pathway in S63del neuropathy may contribute to the identification of novel therapeutic strategies for a broad range of neuropathies characterized by UPR activation.

T10-22A

Oligodendrocyte death in *DTA* mice results in late-onset immune-mediated CNS demyelination

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Multiple sclerosis (MS) is a demyelinating disease characterized by CNS inflammation induced by myelin-specific T lymphocytes. Although MS has long been considered an inflammatory neurodegenerative disease, its etiology is not well understood. The principal 'outside-in' hypothesis for MS pathogenesis centers on the idea that primary dysregulation of the immune system leads to

autoreactivity against myelin-sheath components, which secondarily leads to breakdown of the blood-brain barrier. This is followed by infiltration of the CNS by T cells, leading to the focal inflammation and demyelination that characterize MS lesions. An alternative 'inside-out' hypothesis is based on pathological evidence showing that oligodendrocyte loss and myelin defects occur in the brains of patients with MS even in the absence of apparent signs of inflammation. Thus, the loss of oligodendrocytes and subsequent demyelination might result in autoreactivity against myelin antigens and, secondarily, lead to inflammation and demyelination in the CNS. Here we used the *DTA* (*PLP/CreER^T*;*ROSA26-eGFP-DTA*) mouse model (Traka et al., Brain 2010 Oct;133(10):3017-29) to investigate whether oligodendrocyte death could cause the MS autoimmune response. Upon activation with tamoxifen, *DTA* mice develop widespread CNS demyelination, resulting from pervasive oligodendrocyte loss, that peaks at 5 weeks and resolves by 10 weeks: this is followed by a fatal, secondary demyelinating disease around 40 weeks post-activation. Late-onset disease in *DTA* mice is characterized by focal, MS-like, actively demyelinating lesions that progress to extensive myelin and axonal loss at later disease stages and is associated with increased numbers of activated CD4+ T cells in the CNS and myelin oligodendrocyte glycoprotein (MOG)-specific T cells in peripheral lymphoid organs. Transfer of MOG-specific T cells derived from *DTA* mice to naïve *Rag1*-deficient mice that produce no mature T cells and B cells, resulted in neurological defects that correlated with CNS white matter inflammation. Furthermore, immune tolerization against MOG significantly ameliorated the late-onset disease symptoms in the *DTA* mice. Overall, these data indicate that primary oligodendrocyte death is sufficient to trigger an adaptive autoimmune response against myelin, suggesting that a similar process can occur in the pathogenesis of MS, consistent with the 'inside-out' hypothesis.

T10-23A

Role of Schwann Cell in regulation of myelin sheath properties during nerve fiber excitation and activation of purinergic receptors

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The myelin sheath of nerve fibers of the peripheral nervous system is a sufficiently complex structure consisting of multiple layers of the Schwann cell (SC) membranes. It should be noticed that myelin has been long considered as an electrical insulator serve for rapid nerve impulse propagation in the form of action potential (AP). Indeed, there is evidence that role of myelin in the process of AP propagation is not passive. This study is primary aimed to show how myelin may response on AP generation or SC purinergic receptors activation.

Using such experimental methods as Raman spectroscopy, laser interference microscopy, both allowing non-invasive measurements of live cell, together with fluorescent microscopy, the structural changes in the paranode and the node of Ranvier, a nonmyelinated region of nerve fiber, were investigated. Raman spectroscopy allows studying lipid ordering degree and membrane fluidity directly from lipids and non-directly from carotenoid molecules which are the part of myelin membrane.

Our experimental data shows that different changes in the lipid ordering degree upon nerve excitation in both regions occur: in the paranode it increases, whereas in the node of Ranvier it decreases. The refractive index, obtained by laser interference microscopy, decline in both cases. Interestingly, it was found that such changes in myelin structure are linked to desorption of membrane-bound Ca^{2+}. On the other hand, we revealed an increase in the lipid ordering degree and decrease in myelin membrane fluidity on response to activation of SC purinergic receptors.

Mechanisms of regulation of the myelin membrane fluidity and lipid ordering degree in the functioning nerve fibers will be discussed.

T10-24A

Impaired motor learning as the result of myelin disruption

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Myelin, a multilayered membrane insulation wrapped around the axons, increases axonal conduction velocity at least by 50 fold. Myelination around the axon is thought to be crucial for information processing by changing the timing of neural firing patterns during development and learning. Additionally, Stimulating myelination as a result of impulse activity in axons could enable myelin to be regulated by environmental experience, which could contribute to information processing and learning in the brain. We have demonstrated that local translation of MBP mRNA in oligodendrocyte processes is initialized myelin formation at the site of connection between oligodendrocytes and axons depending on neural activity. These findings provide new insight into how myelination, and thus conduction velocity and function of neural circuits, can be regulated by nervous system activity. Then to consider how activity dependent myelination can be involved in information processing, we used myelin proteolipid protein 1 (PLP1) over expression mouse (PLP-tg). PLP1 is one of the major protein components of the myelin sheath and is thought to have important roles for myelin homeostasis. To understand the neural basis of the cognitive impairment caused by the reduction of the neural conduction velocity, we used two-month-old PLP-tg mice which have a slight reduction of conduction velocity and combined *in vivo*two photon microscopy with a motor learning task. GFP-based Calcium Calmodulin probe (G-CaMP) was induced by an adeno-associated virus (AAV) injection in layer 2/3 of the M1 cortex to enable detecting a difference in the firing pattern of neuronal activity with a lever pulling motor learning task. PLP-tg showed increased spontaneous activity in response to lever pulling motor movement compared with wild type littermates, which associates with a disruption in the timing of synaptic inputs and a performance of the motor learning task. We would like to suggest that reduced axonal conduction velocity results in reduced synchronous activation of neurons within learning circuits to result in impaired motor learning task.

T10-25A

Cdon, a cell surface protein, mediates oligodendrocyte differentiation and myelination

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During central nervous system (CNS) development, oligodendrocyte progenitors (OLPs) establish axonal contacts and multiple branched processes to initiate myelination. Damage to the myelin sheath impairs conduction of electrical impulses and results in neurological disabilities as observed in multiple sclerosis (MS). Remyelination failure in MS has been postulated to be partly due to unsuccessful differentiation of OLPs to mature oligodendrocytes (OLGs). A complete understanding of the molecular signals implicated in cell surface interaction to initiate myelination/remyelination is currently lacking. The objective of our project is to assess whether Cdon, a cell surface protein that was shown to participate in muscle and neuron cell development, is involved in OLG differentiation and myelination. To investigate the functional role of Cdon during OLG differentiation, we overexpressed full length rat *Cdon* gene and performed siRNA knockdown in OLG primary cultures obtained from the cerebral hemispheres of newborn SD rats. The expression levels of myelin lipids and proteins were determined by immunofluorescence and Western Blot analysis. We found that Cdon has a low expression in OLPs, increasing in the early OLGs differentiation stages and decreasing in mature cells. Immunocytochemistry of endogenous Cdon showed its localization on both OLG cell membranes and processes exhibiting varicosity-like structures. Cdon knockdown with siRNA decreased Cdon protein by 62% as well as three myelin specific proteins, MBP, MAG and CNP. Conversely, overexpression of Cdon increased all myelin proteins in OLGs. To further examine the role of Cdon in myelination, we co-cultured OLPs transfected with Cdon siRNA with dorsal root ganglion neurons (DRGNs). After 5 days of culture, we found organized MBP patches were significantly reduced in the Cdon siRNA-treated group. These results suggest that Cdon participates in OLG differentiation and myelination, most likely in the initiation stage of development. This information may provide important clues for the design and development of strategies for the treatment of MS to enhance remyelination and CNS tissue repair. Funded by a studentship and operating grant from the Multiple Sclerosis Society of Canada.

T10-26A

Molecular mechanism of myelin disassembly

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Myelin is a specialized membrane produced by oligodendrocytes, which ensheaths axons enabling fast signal conduction. Myelin is a major target of autoimmune diseases, such as multiple sclerosis. However, the mechanisms of myelin damage and disassembly are poorly understood. Myelin is characterized by the stacking of multiple layers of membranes compacted by myelin basic protein (MBP). We have provided evidence that MBP interaction with the inner leaflet of the myelin bilayer results in charge neutralization and triggers self-association of the protein into larger polymers. Here, we studied the role of MBP in mouse models of demyelinating diseases. We have tested the hypothesis that the transition from the cohesive protein meshwork back into the single soluble components is key to the pathogenesis of myelin disorders. We find that a “reverse” phase transition could be an important mechanism in myelin fragmentation seen in demyelinating diseases. Our data therefore shed new light on the mechanisms of myelin breakdown in diseases such as multiple sclerosis.

T10-27A

Distinct modulation of myelination efficiency by cortical and non-cortical astrocytes

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Multiple sclerosis (MS) is an inflammation-mediated demyelinating disease of the central nervous system, which eventually results in axonal degeneration due to failure of remyelination. Functional remyelination depends, among others, on carefully regulated signalling events, and requires sequential activation, recruitment and maturation of oligodendrocyte progenitor cells (OPCs). In addition to demyelination in the non-cortical areas of the brain, also cortical demyelination has been recognized as a feature of MS pathology. Intriguingly, remyelination is more efficient in cortical MS lesions. Underlying mechanisms of this remarkable difference in remyelination efficiency is likely related to cellular and environmental differences of cortical and non-cortical lesions. Indeed, cortical and non-cortical astrocytes respond differently to local environmental signals. Specifically, *in vitro*, Toll-Like-Receptor (TLR) ligands induce the formation of remyelination-inhibiting fibronectin aggregates, but only by non-cortical and not by cortical astrocytes. In addition to fibronectin, potentially other astrocyte-derived signals might support or impede (re)myelination. Here, we further investigated how cortical, as opposed to non-cortical astrocytes modulate myelination efficiency.

Our data reveal that *in vitro* myelination is more pronounced on a feeding layer of adult cortical than on non-cortical astrocytes. To determine whether the enhanced myelination efficiency by cortical astrocytes was due to secreted factors, extracellular matrix (ECM)-derived factors or cell-cell cues, we examined the effect of these parameters on OPC differentiation and myelin sheet formation. Secreted factors from cortical astrocytes slightly increased myelin-like membrane formation as compared to secreted factors from non-cortical astrocytes, while no effect on OPC differentiation was observed. In addition, secreted factors from cortical astrocytes promoted the viability of cells. No significant differences in OPC maturation were observed, as induced by cell-cell contact and astrocyte deposited ECM-mediated signals, derived from either cortical or non-cortical astrocytes. However, OPC viability was significantly increased in the presence of conditioned-medium obtained from either astrocyte as

compared to normal medium. These findings thus further highlight that cortical astrocytes are more supportive to (re)myelination than non-cortical astrocytes.

T10-28A

Extreme longevity of myelinating oligodendrocytes in mouse

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We have shown that new oligodendrocytes (OLs) continue to be generated in healthy adult mice - even in the 4 month-old optic nerve, in which >98% of axons are already myelinated [1]. This raises the question of whether the late-born OLs and myelin are engaged in remodelling existing myelin (e.g. intercalating among the existing myelin sheaths) or else replacing OLs that die in use. To estimate the survival times of myelinating OLs during healthy adulthood we generated transgenic mice that express iCreER^{T2} under *Opalin* transcriptional control in a bacterial artificial chromosome (*Opalin-iCreER^{T2}*). By crossing these to *Rosa26-YFP* conditional reporters we confirmed that the *Opalin* transgene targets Cre recombination specifically to differentiated OLs; when tamoxifen was administered to double-transgenic offspring at postnatal day 60 (P60) and the mice were analyzed 10 days later, >95% of YFP$^+$ cells in the CNS were also CC1$^+$. When *Opalin-iCreER^{T2}* was crossed to *Tau-mGFP* conditional reporters (mGFP is membrane-tethered) we could visualize differentiated OLs and their myelin internodes in white and grey matter, allowing us to follow the population of mGFP$^+$ myelinating OLs for extended periods after labeling. After 1, 3, 6 or 12 months post-tamoxifen we detected no change in the numbers of mGFP$^+$, CC1$^+$ OLs in the corpus callosum, although the total number of CC1$^+$ OLs had increased after 6 months because of continuous addition of new OLs after P60. Therefore, there is no significant loss of mature OLs over the 1 year time frame that we examined. We are now examining different brain regions including optic nerve to determine whether OL longevity is area-specific. We will also analyze internode numbers and lengths to determine whether the complexity of OLs in different locations alters with age.

1. Young, K.M., Psachoulia, K., Tripathi, R.B., Dunn, S.-J., Cossell, L., Attwell, D., Tohyama, K. and Richardson, W.D. (2013). Oligodendrocyte dynamics in the healthy adult CNS: evidence for myelin remodelling. *Neuron* 77, 873-885.

T10-01B

A key role of the androgen receptor in the sexual dimorphism of myelin

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Both the number of oligodendrocytes and myelin basic protein (MBP)- immunostaining are 20-40% greater in white matter structures of the adult male rodent central nervous system compared to females. This sexual dimorphism is abolished by the castration of males, pointing to a determining role of androgens (Cerghet *et al.*, 2006). In this study, we show that this sexual dimorphism is present as early as postnatal day 10 (P10), corresponding to a time of active myelin formation during development. To examine the role of the androgen receptor (AR), we used a pharmacological approach consisting of daily injections of the selective AR antagonist flutamide into newborn male PLP-EGFP pups (mice expressing the green fluorescent protein under the control of proteolipid protein promoter) until P10. Treatment with flutamide reduced the number of oligodendrocytes and the amount of myelin in striatum and corpus callosum of P10 male pups to their female littermates' levels. To confirm the direct involvement of AR, we used mice with selective inactivation of AR in neurons and macroglial cells of the central nervous system (AR^{NesCre} mice). Immunolabelling with Olig2 (oligodendroglial lineage marker), CC1 (mature oligodendrocyte marker) and MBP was performed on sagittal brain slices of AR^{NesCre} and wild-type mice. At P10, MBP-immunostaining and the number of mature oligodendrocytes co-expressing Olig2 and CC1 were decreased by 15-20% in AR^{NesCre} males compared to wild-type counterparts. Importantly, the difference in Olig2-, CC1- and MBP-immunolabelling between AR^{NesCre} and wild-type males was even more marked in adults (20-30%). The immunohistochemistry results were confirmed by quantitative RT-PCR and Western blotting.

Altogether, our results demonstrate that the intracellular androgen receptor affects the number of oligodendrocytes and the amount of myelin.

T10-02B

Axon path and peripheral nerve structure is altered in the trembler-J mouse model of Charcot Marie Tooth disease

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Bands of Fontana are striations that are visible on the peripheral nerves of mice that appear as bands or spirals travelling through the nerve. Our group previously reported an increase in the frequency of these bands in a model of Charcot Marie Tooth disease type 1A, the trembler-J mouse, concurrent with a relative increase in length of axon per unit length of nerve, when compared to wild type mice (Power et al. 2015). Confocal microscopy revealed that the axons travelling through wild type nerves were closely aligned and followed a sinusoidal path. In our previous paper, axon length was calculated by modelling axon path as a sine wave, and computing path length by applying arc length calculations.

Here we report direct measurements of axon length in wild type and trembler-J mice and we found that axon length in wild type nerves closely matches results previously calculated, but trembler-J nerve fibre lengths calculated using this sine wave model do not accurately reflect directly measured ones, signifying a change in axon pattern. Nerve fibres travelling through trembler-J sciatic nerves were approximately 10% longer than wild type fibres per unit length of nerve, with a mean fibre length to nerve length ratio of 1.191 ± 0.029 in trembler-J nerves compared to a ratio of 1.076 ± 0.01 wild type nerves. Axon paths in trembler-J sciatic nerves were severely disrupted, with smaller amplitudes and wavelengths, jagged and irregular courses, loss of alignment between axons and a higher variability of axon length in each nerve. Axon paths in phrenic nerves were similarly disrupted, however, the pathology did not result in an increased fibre length to nerve length ratio. Wild type phrenic nerves had a fibre length to nerve length ratio of 1.182 ± 0.008, while trembler-J phrenic nerves had a ratio of 1.163 ± 0.022.

The origin of the bands of Fontana is still disputed, however we have confirmed the relationship between axon path and the bands of Fontana by superimposing confocal images of the nerve fibres within nerves on to images of those nerves under oblique illumination. The positions of the bands of Fontana corresponded precisely with the undulating course of nerve fibres.

We have described a novel aspect of trembler-J peripheral nerves, where Schwann cell mutation causes severe disruption to normal axonal path, and reported differences in axon path between wild type sciatic and phrenic nerves. In order to fully understand the trembler-J pathology, it will be necessary to develop a comprehensive understanding of wild type nerve structure in adults and through development.

T10-03B

Demonstration of pyruvate carboxylaxion, pentose phosphate pathway, and mitochondrial activity in cultured oligodendrocytes using ^{13}C-labelled isotopes

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Although oligodendroglia forms a significant proportion of cells in the central nervous system (CNS), their metabolic properties remain poorly understood. Recent studies suggest that mature oligodendrocytes play a fundamental trophic support role to axons via transfer of lactate, rendering these cells as being mostly glycolytic after myelination. However, it is still not known whether additional metabolic pathways significantly contribute to oligodendrocyte bioenergetics and their

potential interactions with CNS astrocytes and neurons. We have therefore sought to characterise other metabolic functions in mature primary rat oligodendrocyte cultures using isotope-labelled substrates. We report that cultured oligodendrocytes have extensive glucose-derived metabolism in the cytosol and mitochondria. Incubation with [1,2-^{13}C]glucose established that the pentose phosphate pathway is highly active in oligodendrocytes with 10% of glucose being metabolised at the phosphoenolpyruvate level. Using [1-^{13}C]lactate or [1,2-^{13}C]glucose as substrates we provide evidence of active anaplerotic pyruvate carboxylation in oligodendrocytes. Moreover, we show that oligodendrocytes are able to convert [1,2-^{13}C]acetate into [1,2-^{13}C]acetyl-CoA and subsequent labelling of TCA cycle intermediates, a function that had only been reported for astrocytes in the CNS. Analysis of labelling patterns of alanine after incubation of cells with [1,2-^{13}C]acetate and [1,2-^{13}C]glucose indicated catabolic pyruvate recycling and acetate acetylation in these cells. In conclusion, we show that oligodendrocytes are a metabolically highly active cell population that may contribute considerably to the metabolic activity of the CNS.

T10-04B

Development of transgenic tools to decipher the role of SOX17 in remyelination

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Multiple sclerosis (MS) is a chronic inflammatory disease of the central nervous system (CNS) which leads to gliosis, axonal damage and focal inflammatory demyelination. Remyelination by the oligodendrocyte progenitor cells (OPCs) occurs as a natural regenerative process in the CNS. Nevertheless, remyelination often fails in MS and this might be due to impaired differentiation of immature oligodendrocytes (OLs). As the mechanisms regulating the OPCs maturation are not well understood, understanding the cellular and molecular mechanisms regulating OPCs differentiation might provide new targets to enhance myelin repair. The transcription factor Sox17 has been identified as a positive regulator of OPCs differentiation. In addition, it has been shown *in vitro* that Sox17 regulates OPCs proliferation and differentiation (1).

The present study aims to further explore the role of Sox17 on oligodendrocyte regeneration and myelin repair, after demyelination in the adulthood. To evaluate the role of Sox17 in myelin repair, two transgenic mouse lines were generated, Sox10rtTA/+::Tet-EGFP as control and Sox10rtTA/+::Tet-Sox17-EGFP in which Sox17 is overexpressed specifically in the OL lineage. To study remyelination profile in these two transgenic strains, we used the toxic lysophosphatidylcholine (LPC) demyelinating model. First of all, we found out that a focal demyelinating lesion by LPC in the spinal cord is necessary to trigger Dox-inducible overexpression of Sox17 in the adult mice. In the control TetGFP, 0.04% of the GFP population expressed Sox17, while in the TetSox17 mice 90% of the GFP$^+$ OLs population expressed Sox17. After validating the model, the pattern of Sox17 overexpression at 7 days post-injection has been assessed by imunohistochemistry labeling using stage specific oligodendroglial markers. Either in TetGFP or TetSox17 mice, Sox17 is rarely expressed in NG2$^+$ OPCs. Moreover, compared to the control TetGFP mice, the percentage of Olig2$^+$/GFP$^+$ and CC1$^+$/GFP$^+$ differentiated OLs is increased from 60% to 90% and from 70 to 96% in the TetSox17 mice. Those results confirm that Sox17 is playing an important role in oligodendrocyte differentiation.

(1) Identification of Sox17 as a transcription factor that regulates oligodendrocyte development. Sohn et al.; The Journal of Neuroscience, September 20, 2006.

T10-05B

Modulation of endocannabinoid signalling and therapeutic effects of MAGL and ABHD6 blockade in the cuprizone model of primary demyelination

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Multiple sclerosis (MS) is a complex inflammatory disease characterized by oligodendrocyte loss and axonal pathology in which lesion formation may be initiated by autoimmune or immune-independent events. Enhancement of brain endocannabinoid function is an accepted strategy for the treatment of MS though the clinical utility of exogenous CB_1 receptor agonists is limited by their potential adverse effects at the cognitive level. Here we analyzed the modulation of endocannabinoid signalling and the therapeutic utility of targeting the hydrolysis of the main endocannabinoid 2-arachidonoylglycerol (2-AG) using an *in vivo* model of primary demyelination that mimics MS pattern III lesions and which consists in the administration of the copper chelator cuprizone in the diet. Demyelination of the corpus callosum (CC) by cuprizone feeding was associated to an increased expression of CB_1 receptors mainly localized to $CD11b^+$ microglia/macrophages, as well as by a marked upregulation of CB_2 receptor gene expression levels. Mice deficient in CB_2 receptor exhibited no differences in the extent of demyelination or presence of $CD11b^+$, $GFAP^+$ or $NG2^+$ cells in the CC during cuprizone administration, suggesting a possible deficit in endocannabinoid tone or signalling that was supported by the increased gene expression of 2-AG hydrolysis enzymes monoacylglycerol lipase (MAGL) and α/β hydrolase domain lipase (ABHD6) in cuprizone-treated mice. Chronic pharmacological inhibition of either MAGL or ABHD6 prevented myelin loss and attenuated the inflammatory response induced by cuprizone feeding as evidenced by a reduced presence of $CD11b^+$ cells in demyelinated CC. The myelin protective effect resulting from MAGL blockade was associated to a reduced recruitment of $NG2^+$ oligodendrocyte progenitors to the CC of mice fed a cuprizone containing diet, an effect that was not mimicked by ABHD6 inhibition. Conversely, inhibition of ABHD6 activity during cuprizone administration was associated to a reduction in the presence of $GFAP^+$ astrocytes in demyelinated CC. These data support the hypothesis that modulation of endocannabinoid signalling participates to the cellular event mediating immune-independent demyelination and support the potential of targeting 2-AG hydrolysis enzymes as novel treatment option in MS.

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T10-06B

The history of myelin

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We can date the first incidence of myelin observation in 18th century by Anthonie van Leeuwenhoek, a draper from Delft in the Netherlands. He reported in a 1717 letter to the British Royal Society: "**Sometimes I observed a nervule…to be completely surrounded by fatty parts… extending here and there from the spinal marrow"**. Thanks to grinding his own lenses, he eventually became an outstanding microscopist. After his death his craft got lost and nothing happened for another century. In 1833, using a new 'Chevalier' microscope, Christian Ehrenberg in Berlin rediscovered myelin in teased nerve fibers. He described myelinated nerves as cylindrical tubes with four parallel lines and named the white matter *medulla*. In 1838, another Berliner Robert Remak distinguished nerve fibers surrounded by medulla with a distinct sheath and nuclei [myelin], from finer transparent fibers with no double border he called *tubulus primitivus* [axon]. In 1854, Rudolf Virchow in Berlin coined the word *myelin* based on the similar appearance of nerve medulla with Greek *myelos* for bone marrow. At the beginning myelin was a floating name. It became strictly associated to myelin only after Max Schultze in Bonn discovered in 1865 that osmium specifically stains it. Soon in 1872, Louis Ranvier in Paris reported the myelin sheath is discontinuous and displays stunning periodic interruptions, quickly named *nodes of Ranvier*. Myelin function remained elusive for a long time and generated wild speculations, although Virchow was the first in 1958 to hypothesize myelin sheath was an electrical insulator. Meanwhile its striking regularity across species triggered a systematic measurement of myelin thickness per axon diameter by Donaldson and Hoke at the University of Chicago in 1905. This was named *g ratio* by Schmitt and Bear in 1937, based on the birefringence optical property of myelin. Ralph Lillie suggested saltatory conduction as early as 1925, which was confirmed twenty years later in 1949 experimentally with complex equations by Huxley and Stämpfli. The lingering question of myelin origin remained a wandering guess until the generalization of electronic microscopy after the Second World War. Betty Geren in Boston in 1954 saw by EM that Schwann cell spirals myelin around the axon. Richard Bunge in 1962 showed the same for oligodendrocyte. It invalidated the pervasive belief, including by Ramon y Cajal, that neuron secretes myelin. Some features such as redundant myelin remain perplexing. The exquisite three-dimensional complexity of myelin continues to defy and drive research.

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T10-07B

A novel role for Endothelin receptor B signalling in the peripheral nervous system

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The endothelin signalling system regulates physiological processes including blood pressure and cell differentiation. Three different endothelins (EDN1-3) have been identified, acting via two different endothelin receptors (EDNR A and B). It has been shown that endothelin receptor type B (EDNRB) controls the development of neural crest cells, which give rise to melanocytes, neurons of the enteric nervous system, dorsal root ganglia and Schwann cells of the peripheral nervous system. Loss of the EDNRB causes congenital aganglionosis of the intestine leading to Hirschsprung's disease in rodents and humans.

In the present study we investigated the role of the endothelin/endothelin receptor B system in peripheral myelination and neurotrophic support of axons provided by Schwann cells. In an *in vitro* assay we treated myelinating dorsal root ganglion (DRG) co-cultures with recombinant endothelin-1 and observed a dramatic reduction of MBP-positive myelin segments. After withdrawal of endothelin-1, cultures immediately started to form myelinated internodes. We conclude from these results, that endothelin signalling reversibly inhibits myelin formation *in vitro*. Moreover, spotting lethal rats (termed *sl/sl*), carrying a homozygous deletion in the EDNRB, showed a higher number of myelinated axons in sciatic nerves at postnatal day (P1) *in vivo,* again pointing to an inhibitory effect of endothelin signalling on myelination. However, at later stages of development, EDNRB-deficient rats developed a hypomyelination predominantly affecting small caliber axons and, finally, loss of small myelinated fibers. Additionally, the total number of Schwann cells was reduced compared to controls. Selective loss of sensory axons has also been observed in neurotrophin 3 (Ntf3) knockout mice. Indeed, we observed a reduction of Ntf3 mRNA in the sciatic nerve of *sl/sl* rats and ENDRB dependent regulation of Ntf3 expression in primary Schwann cells *in vitro*. Our data implicate the endothelin/EDNRB ligand/receptor system as a modulator of myelin formation and neurotrophic support by Schwann cells.

T10-09B

Molecular analysis of the axon initial segment in a cuprizone-induced demyelination model of Multiple Sclerosis

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The axon initial segment (AIS) is a unique axonal domain next to the soma and preceding the myelin sheath in myelinated axons, characterized by a dense clustering of voltage-gated sodium (Nav) and potassium (Kv) channels. The AIS performs two crucial functions: the generation of action potentials (APs) and the maintenance of neuronal polarity. Multiple sclerosis (MS) is a chronic, inflammatory disease of the central nervous system characterized by demyelinating lesions which leads to disorganization of nodes of Ranvier and failure in action potential propagation. Given strong similarities between AISs and nodes and the presence of auto-antibodies against AIS proteins in sera from MS patients, AISs could well be affected, like nodes, in demyelinated conditions like MS. Yet, the AIS has surprisingly never been analyzed in MS. We therefore searched for potential structural and/or molecular alterations at the AIS in a commonly used cuprizone-induced demyelination mouse model of

MS. We initially, measured the length and distance from the soma of the total population of AISs (two crucial parameters known to modulate the neuron's spike generation properties) from different layers of the primary motor cortex in adult C57Bl/6 female mice fed with cuprizone for 6 weeks and compared with control mice (fed with a normal diet). We did not find any statistically significant AIS length or position difference between the two groups of mice. Since complete demyelination is obtained only after five weeks of cuprizone treatment, we also analysed mice fed with cuprizone for 10 weeks. Again we found no significant change in AIS length and position. Given the strong heterogeneity in terms of AIS length within each cortical layer, we decided to focus our analysis on a more specific population of AISs, those from Thy1-GFP-labelled pyramidal neurons within layer V. These Thy-1 GFP labelled cells also might not be equally vulnerable to demyelination as we found 30% of their AISs are not immediately flanked by a myelin sheath.

T10-10B

The oligodendrocyte "processosome": identification of new regulators of differentiation and myelination

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Oligodendrocytes (OL) are the myelinating cells of the central nervous system (CNS). During CNS development and myelination, OL extend several membrane processes that will contact and wrap axons. The formation and extension of such processes requires controlled reorganization of the cytoskeleton.

We physically separated oligodendrocyte precursor cell (OPC) soma from their processes for transcriptomic analysis (RNA sequencing) in order to identify and characterize mRNAs mainly present in the processes of the OPC. We hypothesize that such molecules will play important roles in OL process extension, wrapping and, later, in myelination of axons.

We observed a substantial enrichment of mRNAs in OPC process related to cytoskeletal dynamics and ribosomes/translation, reinforcing the notion of local regulation of translation of mRNAs involved in oligodendrocyte process extension, as it is already described in neurons. We are currently focused on the study of two undescribed molecules, Dusp19 and Kank2, enriched in OPC processes when compared with soma. These participate in important signalling pathways such as JNK-MAPK and Rho GTPases that modulate the cytoskeleton dynamics and are important for OL differentiation. We observed that the expression of these molecules is modulated both in vitro during OL differentiation and in vivo during CNS developmental myelination. Knockdown in vitro experiments suggest key roles in OL differentiation and myelination.

Altogether, this knowledge will contribute to better understand and promote remyelination in debilitating demyelinating diseases such as Multiple Sclerosis.

T10-11B

Lanthionine ketimine ester provides benefit in a mouse model of multiple sclerosis

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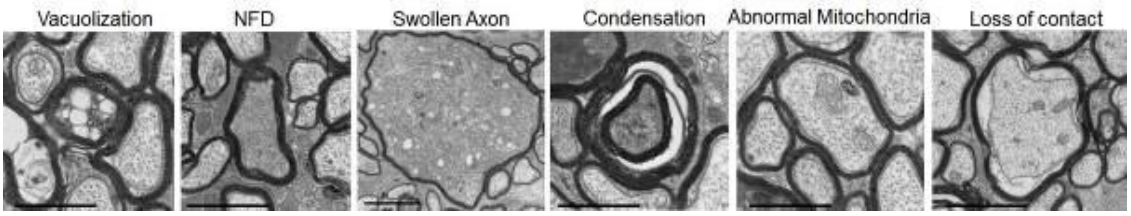
Lanthionine ketimine (LK) is a natural sulfur amino acid metabolite which binds to collapsin response mediator protein-2 (CRMP2), an abundant brain protein that interacts with multiple partners to regulate microtubule dynamics, neurite growth and retraction, axonal transport, and neurotransmitter release. LK ethyl-ester (LKE) is a cell-permeable semi-synthetic derivative that promotes neurogenesis, suppresses nitric oxide production from microglia, and reduces neurotoxicity of microglia-conditioned medium. These properties led us to test the effects of LKE in experimental autoimmune

encephalomyelitis (EAE), a commonly used mouse model of multiple sclerosis. Female C57Bl/6 mice were immunized with myelin oligodendrocyte glycoprotein peptide 35-55 to develop a chronic disease. LKE was provided in the chow at 100 ppm, ad libitum beginning when the mice reached moderate clinical signs. Over the following 4 weeks the LKE-treated mice showed a significant reduction in clinical signs compared to vehicle-treated mice. LKE dose-dependently reduced IFNg production from splenic Tcells, but had no effect on IL-17 production suggesting protective effects were mediated within the CNS. Electron microscopy revealed that, compared to sham mice, EAE mice had significant neurodegeneration in both the optic nerve and spinal cord, which was reduced in the LKE treated mice. In contrast only minimal disruption of myelin was observed at this time point. In the optic nerve, measurements of axon caliber and myelin thickness showed little changes between sham and EAE mice, however treatment with LKE increased the percentage of axons with thicker myelin and with larger axon calibers. In the spinal cord, only smaller effects of LKE on myelin thickness were observed. The effects of LKE were associated with a reduced relative level of phosphorylated CRMP2 to CRMP2. Together, these results demonstrate that LKE reduces neurodegeneration in a chronic EAE model of MS, which could have translation potential for treatment of progressive forms of MS

Image



T10-12B

Gas6/TAM signalling promotes oligodendrocyte differentiation, maturation, and remyelination after toxic injury in culture

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The progressive phase of multiple sclerosis (MS) is associated with blockade of differentiation of oligodendrocyte progenitor cells (OPCs), which would be essential for remyelination. Therefore, one of the key aims of MS therapy is to stimulate OPC differentiation and thereby induce remyelination and repair to damaged axons in the central nervous system (CNS). The TAM family of receptor tyrosine kinases, composed of Tyro3, Axl and Mer, all of which are expressed in the CNS, have been implicated to participate in glial cell development and myelination/remyelination. Their activation by their natural ligands, Gas6 and Protein S, activates signalling pathways that regulate a number of major cellular processes including cell proliferation and survival, phagocytosis and immunomodulation, all of which may be beneficial to the remyelination process in MS. In support of this, Gas6 has been indicated to have both regenerative and immune suppressive functions by both stimulating remyelination as well as suppressing macrophage activation. In this study, we aim to investigate further the effect and mechanisms of Gas6/TAM signalling on oligodendrocyte maturation and remyelination after experimental demyelinating injury in an *in vitro* culture model.

Optic nerves were harvested from 2-month old C57/BL6 mice and treated with Gas6 for 72 hours in culture to analyse changes in protein expression by western blotting. Also, cultured cerebellar slices from mice at postnatal day 10 were treated with lysolecithin as a toxic non-immune model of demyelination *in vitro* to study the effect of Gas6 on such injury. Furthermore, Gas6 was incubated with isolated human OPCs in culture and their differentiation was monitored under fluorescence microscopy. Our optic nerve culture studies revealed that Gas6 significantly increased the expression of myelin-basic protein (MBP) after 3 days incubation in culture. In cultured cerebellar slices, co-incubation with Gas6 attenuated the level of demyelination induced by lysolecithin, as observed through MBP staining intensity and extent. We are currently completing the experiments on the effect of exogenous Gas6 on human OPC differentiation in culture.

In conclusion, we have shown in our various culture models that Gas6/TAM signalling stimulates production of MBP in CNS white matter, and consequently remyelination in the brain after toxic injury. The signalling mechanisms behind these cellular observations are currently under investigation. This work is funded by the MS Society UK.

T10-13B

The nootropic agent nefiracetam enhances myelin repair

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Multiple sclerosis (MS) is an autoimmune disease and the current therapies, all immune suppressants, carry inherent risks and are relatively ineffective in treating progressive disease forms. Myelin repair offers an alternative target for intervention, distinct and possibly complementary to immune modulation. The mechanism governing repair, migration and maturation of oligodendrocytes during recovery is still relatively poorly understood, making this a difficult target to exploit for drug development despite the tremendous potential of this approach. Here, we report our data on nefiracetam, a compound never previously associated with myelin biology, which suggests that this compound can accelerate the spontaneous rate of myelin repair, both in vitro and in vivo. We show that nefiracetam can mediate myelin repair in the lysophosphatidylcholine (LPC)-induced myelin toxicity in vitro model in organotypic hippocampal slice culture across a range of concentrations. In this in vitro model, nefiracetam clearly augments repair of myelin following damage rather than protection against such lesions. Nefiracetam can mediate myelin repair in vivo through a non-inflammatory mechanism in the cuprizone diet model of demyelination. In this study, treatment with nefiracetam mediated a significantly improved recovery of myelin in the corpus callosal pathway. We have also confirmed that, in the classic MS in vivo model of EAE, if inflammation is controlled by the steroid anti-inflammatory dexamethasone, then nefiracetam can alleviate symptoms of motor impairment. Additionally, we show that nefiracetam can promote oligodendrocyte maturation in purified oligodendrocyte precursor cell culture. These data strongly support nefiracetam as a potential novel therapy that could significantly improve treatment of MS.

T10-14B

CNS-pericytes promote oligodendrocyte fate decision and differentiation contributing to myelin development and repair

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Multiple sclerosis (MS) is the most frequent demyelinating disease in young humans with over 2 Mio people affected. Although remyelination is a robust response upon demyelination, it largely fails in more advanced stages. The endogenous cellular sources of remyelinating oligodendrocytes are oligodendroglial progenitor cells (OPCs), widely distributed throughout the central nervous system (CNS). In response to demyelination, several cellular and molecular cues regulate OPC activation,

proliferation, recruitment and differentiation. However, the current knowledge about CNS remyelination is barely known and a better understanding represents a crucial step towards developing future MS therapies. Although angiogenesis and blood-derived elements participate in myelin repair, it is not much known about the role of the neurovascular niche (NVN) during remyelination. We have previously shown that mesenchymal stem cells (MSCs) induce neural stem/progenitor cells (NPCs) and OPCs towards an oligodendroglial fate and thus MSC therapy has been proposed for MS treatment. As part of the NVN, CNS-resident pericytes (PCs) regulate vascular homeostasis and blood-brain-barrier integrity. Besides this, PCs share several features with MSCs and are in close contact with CNS progenitors, thus, we hypothesize that PCs might influence OPCs response to demyelination and participate in myelin repair. We observed that upon focal-induced demyelination PCs activate, proliferate, migrate away from vessels and change their morphology. Furthermore, we found that the distance between PCs and OPCs is specifically shortened during the course of remyelination and the proportion of differentiated oligodendrocytes increases within the PC proximities. *In vitro* we demonstrated that PC-derived conditioned medium instructs oligodendroglial fate decision in adult NPCs and leads to an increased differentiation and survival in OPCs. Moreover, we confirmed that PC-derived soluble factors enhance remyelination in *ex vivo* demyelinated cerebellar slice cultures. Finally, we found that PC-deficient mice display a developmental delay in postnatal oligodendrogenesis and myelination within the spinal cord and the optic nerve. Taken together our data indicate that PC-derived soluble factors induce oligodendrogenesis in CNS-progenitors and suggest that PCs contribute to oligodendrocyte development, myelination and myelin repair.

T10-15B

In vivo and *in vitro* evaluation of MAGL and ABHD6 as novel therapeutic targets in multiple sclerosis

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Activation of cannabinoid CB_1/CB_2 receptors is considered a useful therapeutic strategy for the treatment of demyelinating inflammatory disorders such as multiple sclerosis (MS) based on the beneficial effects of cannabinoid agonists in experimental models of the disease. The clinical utility of exogenous cannabinoids is however limited by the appearance of unwanted responses related to memory and learning impairment. Recent evidence indicates that blocking the enzymatic metabolism of the main endocannabinoid 2-arachidonoylglycerol (2-AG) may engage both cannabinoid receptor-dependent and -independent therapeutic benefits against neuroinflammation whilst limiting adverse effects. Here we have evaluated the potential of targeting the 2-AG hydrolytic enzymes monoacylglycerol lipase -MAGL- and α/β hydrolase domain lipase -ABHD6- as novel treatment option in MS. Chronic administration of the MAGL inhibitor JZL184 ameliorated neurological deficits during the course of experimental autoimmune encephalomyelitis -EAE- in a dose dependent manner. The beneficial effects of JZL184 at the motor level were associated to attenuated myelin loss and inflammation in the spinal cord white matter of EAE mice, but also to an impaired coupling ability of brain cannabinoid receptors to $G_{i/o}$ proteins. Pharmacological inhibition of ABHD6 ameliorated neurological disability during EAE with the brain permeable compound KT182 being more effective than the peripherally restricted KT203. The ability of both compounds to attenuate inflammation in the brain and spinal cord of EAE mice is currently under investigation.

Incubation with JZL184 or 2-AG attenuated AMPA induced cytotoxicity in cultured oligodendrocytes, an effect that was associated to a reduced Ca^{2+} overload and mitochondrial depolarization. Protection from excitotoxicity by MAGL blockade was mediated through the activation of CB_1 receptors expressed by oligodendrocytes and was not mimicked by the ABHD6 inhibitor KT182, pointing to a predominant role for MAGL in the regulation of 2-AG metabolism in this cell type. Our findings suggest that blockade of MAGL and ABHD6 engages different cellular mechanisms *in vivo* and support the utility of 2-AG hydrolysis inhibitors for therapeutic intervention in MS.

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T10-16B

An Egr2 long antisense-RNA regulates peripheral myelination

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Egr2 is a central regulator of Schwann cell myelination, and *Egr2* loss of function mutations have been associated with several types of human peripheral neuropathies. Following peripheral nerve injury, Egr2 expression is acutely downregulated resulting in initiation of demyelination, which is the initial step of Wallerian degeneration, and ultimately leads to nerve regeneration. However, the role of epigenetic mechanisms, which regulate *Egr2* expression in the peripheral nervous system (PNS) during nerve injury response have not been established. Here we demonstrate the presence and role of a natural occurring long antisense RNA that targets the *Egr2* proximal promoter in myelinating Schwann cells. *In situ* hybridization shows that the Egr2 AS-RNA is expressed during development of the PNS at the time of Schwann cell migration from the neural crest and during Schwann cell maturation. We discovered that peripheral nerve injury induces the expression of the *Egr2* antisense RNA and the downregulation of the *Egr2* RNA and proteins. Infection of myelinated Dorsal Root Ganglion (DRG) explant cultures with a lentivirus expressing the *Egr2* antisense RNA results in significant demyelination as quantified by Myelin Basic Protein (MBP) expression. Lentivirus infection of DRG cultures with the Egr2 AS-RNA before the onset of myelination results in reduced myelination levels. In addition, directed repression of the RNA antisense by LNA (locked nucleic acid)-DNA GapMer antisense oligonucleotides reverts the effect of the *Egr2* AS-RNA-induced demyelination on the DRG cultures. Finally, using RNA-ChIP we found an association of the Egr2 AS-RNA with Argonaute 1, 2 and tri-methyl histone H3 in sciatic nerves. Following sciatic nerve injury the Egr2 AS-RNA preferentially associates with Argonaute-2 in the presence of chromatin repressive marks. Our results suggest that a long non-coding AS-RNA modulates the transcription of *Egr2* and regulates demyelination during nerve injury response through the formation of epigenetic silencing complexes with Argonaute-2.

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T10-17B

***De novo* synthesis of fatty acids in oligodendrocytes is critical for CNS myelination**

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Schwann cells in the peripheral and oligodendrocytes in the central nervous system require a tremendous amount of lipids towards myelin membrane production. Furthermore, when compared to other cells, myelin membrane presents an extremely high lipid content (~80% of dry weight) coupled to an unique lipid composition. Among the main represented class of lipids in myelin are phospholipids and cerebrosides, of which fatty acids represent the building blocks. Most cells preferentially uptake circulating free fatty acids. However, cells under increased metabolic demand, i.e. highly proliferating stem and cancer cells, functionally rely upon their endogenous synthesis via the enzyme Fatty acid synthase (Fasn). Due to their extreme increased membrane production, membrane high lipid-content, and specific relative lipid composition, myelinating cells are liable to functionally depend upon endogenous fatty acids synthesis, instead of solely on their uptake. In this study, we addressed the functional role of *de novo* fatty acid synthesis in central nervous system (CNS) developmental myelination. We analyzed Olig2Cre Fasn floxed mice and show a novel critical function for *de novo* fatty acid synthesis in oligodendrocytes towards fully functional CNS myelination *in vivo*.

T10-18B

Nefiracetam is ineffective in reversing myelin damage in the trembler-J model of Charcot Marie Tooth disease

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Our laboratory has recently identified nefiracetam as a compound capable of accelerating remyelination (Murphy et al, 2013), but the molecular mechanism underlying this effect remains to be elucidated. Given the complex pharmacology of the drug, one strategy to assess mechanism is to investigate its efficacy in repair of peripheral myelin. Some of the biology underlying myelin development and remyelination is known to be common to the central and peripheral nervous systems, whereas other factors appear to be specific to one system or other. Therefore, determining whether the drug is effective in the peripheral nervous system should narrow the list of potential mechanisms of action, regardless of the outcome.

Adult trembler-J mice were treated with 30mg/kg nefiracetam for a period of 30 days. At the end of the treatment period, motor function was assessed with rotarod testing. While trembler-J mice had impaired performance in the rotarod test, nefiracetam treated mice showed no difference in performance when compared to vehicle treated mice. Nerve conduction studies in isolated sciatic nerves post mortem also showed no differences in conduction between nefiracetam and vehicle treated mice. Histological analysis of the sciatic nerves showed no differences in myelin thickness, axon calibre, or g-ratio between nefiracetam and vehicle treated animals, and the axon elongation characteristic of the trembler-J phenotype (Power et al, 2015) was also unchanged between treatment groups. However, the increase in density of nodes of Ranvier seen in the trembler-J mice, reflecting decreased intermodal distance, was reversed by nefiracetam treatment, suggesting there may be a direct effect of the drug on Schwann cells. Nonetheless, the balance of evidence does not suggest that nefiracetam has efficacy in the peripheral nervous system. Therefore, we cautiously conclude that the mechanism of action of nefiracetam in accelerating remyelination is dependent on a molecular target specific to CNS myelin.

Murphy RP, Keogh EA, O'Shea SD, Bowen G, Pickering M, Murphy KJ (2013). Glia 61: S134-S135

Power BJ, O'Reilly G, Murphy RP, Murphy KJ, Pickering M, Jones JFX (2015) Muscle & Nerve 51:246-252

T10-19B

Non-coding RNAs in the differentiation of oligodendrocyte precursor cells

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Non-coding RNAs (ncRNAs) are important regulators of cell fate and have been described to have a higher tissue specificity than protein coding RNAs. Oligodendrocytes (OL) are neuroepithelium-derived cells that insulate neuronal axons through myelin-containing membranes, essential for axonal integrity and impulse transmission. OL precursor cells (OPCs) start to be specified early during embryogenesis but regardless of the origin, their terminal differentiation and functional maturation occurs only at post-natal stages. Non-coding RNAs are important regulators of chromatin status and might play key roles in regulating and maintaining transcriptional programs that govern OL development. This project involves the identification of key ncRNAs involved in the transition between major epigenetic states of OL cells. Through bulk total RNA sequencing on FACS sorted OPCs and single cell RNA sequencing on mouse cortex and hippocampus, we obtained a list of ncRNAs candidates that could modulate OPC differentiation. We are investigating their role in OPC differentiation by techniques such as knock down or over-expression along with their mechanism of action.

T10-20B

Proper myelin maturation during postnatal development depends on Apolipoprotein D function

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Apolipoprotein D (ApoD) is a Lipocalin expressed by glial cells during development, adulthood and aging of the vertebrate nervous system. In the peripheral nervous system (PNS) it is secreted by Schwann cells, while astrocytes and oligodendrocytes are the source of ApoD in the CNS. ApoD shows neuroprotective functions in response to oxidative stress, Wallerian degeneration and aging. We have demonstrated that ApoD controls the dynamics of post-injury myelin recognition and degradation in the PNS of adult and aged mice. Also, nerve conduction velocity is diminished in ApoD-KO mice. These effects are accompanied by genotype-dependent differences in myelin composition. Is ApoD altering myelin structure-function from early on in development, and thus, conditioning the response to injury in adult and aged mice?

To investigate this question we have performed a molecular profile and an electron microscopy analysis of ApoD-KO and wild-type mice from postnatal development to aging (3 days - 21 months). While early in development the initial phases of myelination take place correctly, both CNS and PNS myelin from ApoD-KO mice show abnormal periodicity and defective myelin compaction at 90 days. MBP and MAG protein profiles strongly support that lipid compaction is delayed in the absence of ApoD, and lipid analysis of ApoD-KO myelin shows altered phospholipid composition, particularly in phosphoinositid species, important for the interaction of MBP with myelin membranes in the process of compaction.

Our results demonstrate that ApoD function is relevant for an adequate myelin membrane compaction, a process where lipid-protein interactions have to be orchestrated in order to construct a proper electrically insulating layer. The presence of ApoD in myelin membranes from early periods of development is therefore required to construct nerves with adequate conduction properties and an adaptive response to injury.

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T10-21B

Regulatory role of the thrombin receptor in myelination

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Hemorrhagic white matter injuries in the perinatal period are a growing cause of cerebral palsy yet no neuroprotective strategies exist to prevent the devastating motor and cognitive deficits that ensue. In addition to its role in the coagulation cascade, thrombin directs discrete cellular actions by N-terminal cleavage of a seven transmembrane G-protein coupled receptor, protease activated receptor 1 (PAR1), also referred to as the thrombin receptor. We recently demonstrated a unique role for PAR1 activation in suppressing myelin gene expression, in limiting oligodendrocyte progenitor (OPC) process elaboration, and in exacerbating the impact of oligotoxic agents *in vitro* (Burda et al., 2013). Here we show that the thrombin receptor plays a critical role in the development of spinal cord myelination. Specifically, PAR1 gene deletion resulted in earlier onset of spinal cord myelination, including substantially more Olig2-positive oligodendrocytes, more myelinated axons and higher proteolipid protein (PLP) levels at birth. Enhancements in myelination associated with PAR1 gene deletion were also observed in adulthood as evidenced by higher amounts of myelin basic protein (MBP) and thickened myelin sheaths. Enhancements in myelination in the spinal cord as a result of

PAR1 loss-of-function were associated with elevations in the activated forms of the pro-myelination signaling intermediates ERK1/2 and AKT. Nocturnal ambulation and rearing activity were also elevated in PAR1-/ mice. An integral role for PAR1 as a suppressor of pro-myelination events is supported by findings demonstrating the highest levels of PAR1 expression in the spinal cord occur at birth with substantial reductions during the first postnatal week with the onset of myelination. *In vitro*, the highest levels of PAR1 were observed in OPCs, being reduced with differentiation. In parallel, the expression of PLP and MBP, in addition to Olig2, were all significantly higher in cultures of PAR1-/- oligodendroglia. Moreover, application of a small molecule inhibitor of PAR1 to OPCs *in vitro*, increased PLP and MBP gene expression. Together these findings suggest that the thrombin receptor is an integral biological translator of microenvironmental protease activity that can directly impact myelin biogenesis. Future studies will be needed to determine whether this powerful extracellular regulatory switch can be targeted therapeutically to improve myelin production in the context of hemorrhagic white matter injury or disease. Supported by NIH R01NS052741 and NMSS RG4958A5/1

T10-22B

Aquaporin 1 is localized in the Schmidt-Lanterman incisures and at the paranodes of the nodes of Ranvier in the rat sciatic nerve

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Aquaporins (AQPs) are a family of small, integral membrane water-transporting proteins, found in prokaryotes and eukaryotes implicated in mediating bidirectional movement of water across cell membranes in response to osmotic gradients. There are at least 13 different members of the AQP protein family described in mammals. In the nervous system, most of the work has been focused in the central nervous system, but very little in the peripheral nervous system (PNS). In the PNS, AQP1, AQP2 and AQP4 have been reported in both peripheral neurons and glial cells. In this work we studied the expression of four AQPs (AQP1, AQP2, AQP4 and AQP9) by reverse transcription polymerase chain reaction (RT-PCR), showing that only AQP1 is present in the sciatic nerve. AQP1 is also observed at the protein level by Western blot analysis. We also studied the localization of AQP1 in the sciatic nerve by immunohistochemistry. The results show that AQP1 is present in both myelinating and non-myelinating Schwann cells. The expression of AQP1 in non-myelinating Schwann cells supports the involvement of AQP1 in pain perception. In myelin internodes AQP1 is enriched in the Schmidt-Lanterman incisures and in some internodes it is also present in the abaxonal membrane. AQP1 is also present in the paranodal regions of the nodes of Ranvier, which co-localizes with actin. Therefore, AQP1 might play an important role in myelin homeostasis maintaining the thermodynamic equilibrium across the plasma membrane in myelinated axons during electrical activity.

T10-24B

The role of endothelin signalling in myelination

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Myelination, the process of axonal wrapping by oligodendrocytes, is a tightly regulated programme. Endothelin-1 and endothelin-2 have recently been shown to incur conflicting outcomes on myelination both acting via endothelin receptor B (EDNRB). Here we resolve this conflict through manipulation of the EDNRB signalling pathway. Cultures of rat oligodendrocytes on synthetic microfibers regulate the number of myelin sheaths they produce under the influence of EDNRB and downstream SRC-family kinases, with no effect on sheath length. In vivo, EDNRB mutant zebrafish develop more oligodendrocytes however mosaic labelling of individual cells demonstrated that they produce fewer myelin sheaths. From these results we propose a model whereby EDNRB regulates different stages of myelination through opposing distinct mechanisms potentially linking myelin sheath formation to vasculature supply.

T10-25B

The role of fibroblast growth factor 9 in multiple sclerosis: inhibition of myelination and induction of pro-inflammatory environment

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Remyelination failure plays an important role in the pathophysiology of multiple sclerosis (MS), but the underlying cellular and molecular mechanisms remain poorly understood. We now report expression of fibroblast growth factor 9 (FGF9) is not only up regulated in early active MS lesions, but remains elevated at sites of ongoing tissue damage in patients with longstanding progressive disease. *In vitro* we could demonstrate that FGF9 impedes myelination and remyelination, which is associated with the appearance of multi-branched "pre-myelinating" MBP^+/PLP^+ oligodendrocytes that interact with axons but fail to assemble myelin sheaths; an oligodendrocyte phenotype described previously in chronically demyelinated MS lesions. The effect of FGF9 is mediated by binding to its high affinity receptors (FGFR2 and FGFR3) that are expressed by many cells in the CNS including astrocytes, oligodendrocyte precursors (OPC) and myelinating oligodendrocytes. In isolated OPCs FGF9 inhibits their differentiation and it acts as a mitogen on mature oligodendrocytes, but this direct effect alone might not be responsible for its ability to inhibit myelination in the far more complex, multicellular environment of our myelinating cultures. In addition, we could show that the inhibitory activity of FGF9 is partly mediated by factors secreted by astrocytes. Transcriptional profiling and functional validation studies demonstrate these include a major contribution from tissue inhibitor of metalloproteinase (TIMP)-sensitive proteases, implicated in extracellular matrix remodelling, in particular a disintegrin and metalloproteinase with thrombospondin type 1 motif (ADAMTS) and matrix metalloproteinase (MMP) family members. However, this effect on (re)myelination does not occur in isolation but is accompanied by increased expression of *Ccl2* and *Ccl7*, two pro-inflammatory chemokines that contribute to recruitment of microglia and macrophages into MS lesions. These data indicate glial expression of FGF9 can initiate a complex astrocyte-dependent response that contributes to two distinct pathogenic pathways involved in the development of MS lesions. Namely, induction of a pro-inflammatory environment and failure of remyelination; a combination of effects predicted to exacerbate axonal injury and loss in MS patients.

T10-26B

L-PGDS/GPR44: new regulators of Peripheral Nervous System myelination

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Myelin, the multilamellar spiral membrane surrounding the axons, is synthesized in the peripheral nervous system (PNS) by Schwann cells (SC). The key signal for PNS myelination is axonal Neuregulin 1 (NRG1) type III, whose activity is controlled by proteases mediated extracellular cleavage. We showed that in sensory neurons NRG1 type III intracellular domain is cleaved by the γ-secretase complex and that the generated fragment translocates into the nucleus to up-regulate the prostaglandin D2 synthase (L-PGDS) gene, thus undergoing a classical regulated intramembrane proteolysis. L-PGDS catalyzes the enzymatic conversion of prostaglandin H2 (PGH2) to prostaglandin D2 (PGD2), one of the major lipid mediators synthesized in the nervous system.

We demonstrated that L-PGDS is secreted by PNS neurons. In addition ablation of L-PGDS or inhibition of its enzymatic activity in vitro impairs myelination in neurons-SC myelinating co-cultures and causes myelin alterations. Accordingly, myelin in sciatic nerves of L-PGDS null mice is noticeably

thinner and aberrant. We also showed that the PGD2 receptor GPR44 is the most expressed in SC and that its specific knock down impairs myelination in vitro and in vivo.

In conclusion, our studies show that L-PGDS, PGD2 and GPR44 are new regulators of PNS myelination and maintenance and their modulation could be beneficial for the treatment of demyelinating neuropathies.

T10-27B

CNS myelin and axon morphology in demyelination and dysmyelination in mouse models

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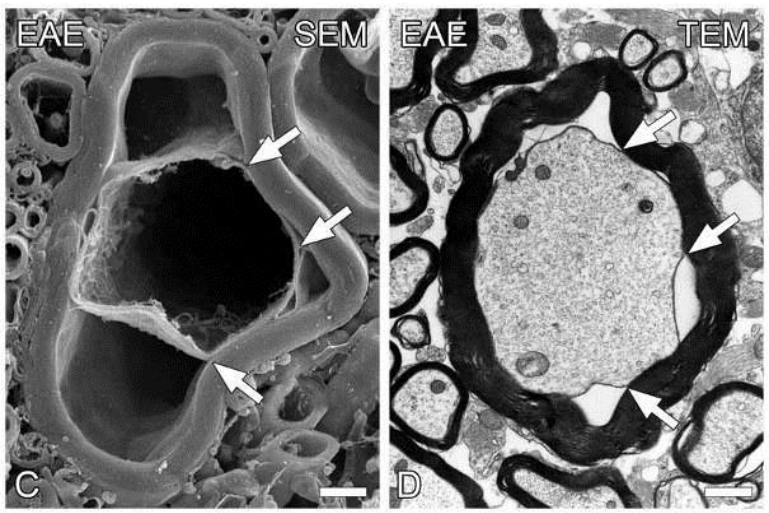
Demyelination and dysmyelination are observed in demyelinating diseases such as multiple sclerosis (MS). However, it has not been fully understood the pathological changes of these morphologies. To address this issue, myelin basic protein-deficient *shiverer* mice and mice with experimental autoimmune encephalomyelitis (EAE) were examined how the morphological changes of myelin and the axon were induced by dysmyelination and demyelination.

Scanning electron microscopy (SEM) with an osmium-macerated method enabled to observe the three-dimensional membranous structures such as myelin and the axon in the spinal cord. SEM images showed dramatic ultrastructural abnormality of myelin and the axon in the white matter of spinal cord during EAE (Figure). For example, myelin detachment and excess myelin formation were observed as typical abnormalities of myelin during demyelination in EAE. Importantly, compact myelin was well preserved even though in these situations. SEM images also showed the morphological changes of axonal intracellular organelles during demyelination and dysmyelination. Enriched mitochondria and well-developed sER in the axons were observed in *shiverer* mice. Similar to this observation, axonal morphological changes of mitochondria and sER were also observed in mice during EAE. In addition, we show that the estimation of demyelination by light microscopic techniques such as luxol fast blue staining is not always suitable using sequential sectioning of the lesion of EAE. These observations indicate that there is a close relationship between myelin and axon morphology. In addition, it will be considerable that there is some signaling between myelin and the axon.

Taken together, this technique will be powerful tool for underlying the mechanism of the pathogenesis in demyelination and axonal degeneration.

Image



Poster topic 11
Neural stem/progenitor cells

T11-01A

Molecular and ultrastructural alterations of the neural stem cells from dystrophic mdx mouse

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X-linked chromosome mutations resulting in the absence of the dystrophin protein cause Duchenne muscular dystrophy (DMD) in humans and in dystrophic mdx mouse. DMD is characterized by progressive muscle degeneration, neural dysfunctions and mental retardation. Dystrophin, an actin binding protein which links the cortical actin of the myofibers to the sarcolemma, has several truncated isoforms including the Dp71, which have been demonstrated in the perivascular astrocytes forming the blood-brain barrier (BBB). The full-length dystrophin and the Dp71 are linked to the dystrophin associated proteins (DAPs), including the water channel aquaporin-4 (AQP4), dystroglicans, sarcoglycans, dystrobrevins and syntrophins, forming a protein complex which connects the extracellular matrix to the cytoskeleton providing structural BBB stability.
We previously reported a deficiency in the Dp71 and DAPs proteins in the mdx perivascular glial endfeet and myofibers coupled with BBB opening and oedema, indicating a key role for DAPs complex in the vascular alterations occurring in this pathological condition.
In this study, we isolated for the first time from the brain of the mdx mice, the adult neural stem cells (ANSCs) and characterized them by FACS and electron microscopy. Moreover, we studied the expression of the DAPs AQP4, potassium channel Kir4.1, α- and β-dystroglycan (α/βDG), α-syntrophin (αSyn), glial fibrillary acidic protein (GFAP) and Dp71 by confocal laser scanning microscopy, real time-PCR and western blotting.
The results showed that the mdx ANSCs expressing the CD133, Nestin and Notch receptor were reduced in the number compared to controls, they showed a retard in the cell cycle, and ultrastructurally, they appeared 50% size reduced with a few cytoplasmic organelles compared to control ones. Furthermore, numerous apoptotic cells were recognizable in the mdx neurospheres. We also demonstrated that glial precursor stem cells, likewise the differentiated glial cells, already expressed Dp71 and the DAPs protein in a membrane polarization in the control brain. Instead, a strong post-transcriptional reduction and molecular membrane disarrangement of the Dp71 and DAPs proteins was present in the mdx ANSCs. Overall, these results suggest that the increased apoptosis and the deficiency in Dp71 and DAPs proteins in the mdx ANSCs could induce altered differentiation into glial cells in turn responsible of the BBB injuries present in the adult dystrophic brain.

T11-02A

Mitochondrial dysfunction mimics the impact of ageing on hippocampal neurogenesis

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During adulthood, a specialized population of astrocytes with a radial glia-like morphology serves as neural stem cells in the hippocampus and generate dentate granule neurons throughout adulthood, which fulfill essential function in hippocampal information processing. Maintenance of the stem cell

pool has to be tightly regulated to sustain hippocampal neurogenesis throughout life, nevertheless, the stem cell pool shrinks and is functionally compromised during ageing. Here, we investigated whether mitochondrial dysfunction - a potential player in organismal ageing - affects hippocampal neurogenesis. Conditional deletion of TFAM (essential for the transcription of respiratory chain proteins encoded by mtDNA) in neural stem cells and astrocytes is sufficient to mimic the impact of ageing on hippocampal neurogenesis and results in decreased proliferation, enhanced glial differentiation, and impaired survival and maturation of newly generated neurons. Intriguingly, enhancing mitochondrial respiration ameliorates the age-associated neurogenesis effects. These data indicate that mitochondrial dysfunction in neural stem cells may at least in part underlie age-associated neurogenesis defects and suggest mitochondrial function as a potential target to restore stem cell performance and neurogenesis in the ageing hippocampus.

T11-03A

Activation of Adenosine A1 Receptor shifts neural stem cells fate from neurogenesis to astrogliogenesis

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Toxicity of extracellular purines is among the factors inhibiting adult neurogenesis during neurodegenerative diseases. After neurodegeneration extracellular ATP and adenosine are released at high concentrations and alter the homeostasis and survival of glia and neurons. In this study we examined the effects of adenosine in modulating the cellular fate of adult neural stem cells from the rat subventricular zone. We observed by immunofluorescence and cytofluorimetry that high concentrations of adenosine (100mM) promote astrogliogenesis at the expense of neurogenesis. Although all adenosine receptors (A1, A2a, A2b and A3) are expressed in these cells, we found that only A1 is involved in the inhibition of neuronal differentiation, as demonstrated by qRT-PCR, Western blot and specific gene silencing. Furthermore, activation of A1 receptor induced the downregulation of a family genes involved of neurogenesis as demonstrated by genomic analysis. In turn, we found that the mechanisms by which adenosine inhibits neuronal differentiation and sustains astrogliogenesis involves the release of IL10 and further activation of the Bmp2/SMAD3 pathway. In vitro data were confirmed in in vivo experiments using intracerebroventricular infusion of the A1 agonist CPA that showed a drastic reduction of neurogenesis and a parallel increase of astrogliogenesis in the olfactory bulb of adult rats. These data further supports the idea that purinergic signaling contributes to the regulation of adult neurogenesis, especially in pathological conditions when purines are present at high concentrations in the extracellular space. In addition, our findings reveal a critical role of IL10 in balancing the differentiation of neural stem cells from the subventricular zone into neurons or astrocytes.

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T11-04A

Implementation of the stem cell properties of NG2+ cells: focus on the epigenetic modulator VPA and the purinergic receptor GPR17

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NG2-expressing parenchymal precursors represent the vast majority of proliferating cells in the healthy adult brain. Normally they give raise to mature oligodendrocytes during development and adulthood. However, it has been also suggested that, under some conditions, NG2+ cells retain stem cell potential and can generate astrocytes and neurons as well.
Previous studies from our laboratory have identified the purinergic receptor GPR17 as a new marker of early stages of NG2+ cell differentiation, showing that GPR17 activation accelerates the oligodendrocyte fate of NG2 cells.
Based on these premises, aim of this work was to unveil the stem cell properties of NG2+ precursor cells and to induce their differentiation towards the neuronal lineage, also through the modulation of

the GPR17 receptor.
Primary NG2+ cells derived from rat cerebral cortex have been cultured according to two published protocols (Kondo and Raff, Science 2000, 289:1754-57; Liu et al., J Neurosci 2007, 27:7339-43), both able to unveil their stem cell properties. In both protocols, we verified if and how exposure to the anticonvulsant agent valproic acid (VPA, 500 μM) can modulate NG2+ cell plasticity and their differentiation to neurons. In fact, VPA is known as an epigenetic modulator, which inhibits histone deacethylases (HDAC) and consequently induces transcriptional changes which favor neurogenesis. In both culturing protocols, we observed an increase in the percentage of cells expressing the neuronal marker β-tubulinIII (βtubIII) upon treatment with VPA compared to control. Interestingly, GPR17, which is normally expressed only by NG2+ cells, was also surprisingly detected in a subset of βtubIII+ cells already under control differentiative condition, suggesting its potential involvement in neurogenesis. The appearance of this cell population was further incremented by exposure to VPA. Taken together, our results suggest that exposure to VPA selects a population of "hybrid" NG2+ cells with an oligodendrocyte morphology that starts acquiring neuronal proprieties. We are currently testing whether the pharmacological modulation of GPR17 receptor can further favor the differentiation towards neurons of GPR17/βIII-tub double-positive cells.
Acknowledgments: Partially supported by NEUROREPAIR project (Dote Ricerca Applicata, Regione Lombardia/Sanofi-Aventis).

T11-05A

Emx2* expression levels in NSCs modulate astrogenesis rates by regulating EgfR and *Fgf9

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Cortico-cerebral astrocytogenesis is a tightly regulated process. It initiates at low level in the middle of neuronogenesis and peaks up after its completion. Astrocytic outputs depend on two primary factors: progression of multipotent precursors towards the astroglial lineage and sizing of the astrogenic proliferating pool. The aim of this study was to investigate the role of the *Emx2* gene in both processes.

We addressed this issue by combined gain- and loss-of-function methods. Tests were run in vivo as well as in primary cultures of cortico-cerebral precursors. We found that *Emx2* overexpression in cortico-cerebral stem cells shrinked the proliferating astrogenic pool, resulting in a severe reduction of the astroglial outcome. We showed that this was caused by EgfR and *Fgf9* downregulation and that both phenomena originated from exaggerated Bmp signalling and *Sox2* repression. Finally, we provided evidence that in vivo temporal progression of *Emx2* levels in cortico-cerebral multipotent precursors contributes to confine the bulk of astrogenesis to postnatal life.

Emx2 regulation of astrogenesis adds to a number of earlier developmental processes mastered by this gene. It points to *Emx2* as a new promising tool for controlling reactive astrogliosis and optimizing cell-based designs for brain repair.

T11-06A

Activation of NFAT transcription factors in neural precursor cells induces astrocyte and neuron differentiation

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The study of factors that regulate the survival and differentiation of neural precursor cells (NPCs) is an essential step to understand neural development as well as brain regeneration. The Nuclear Factor of Activated T Cells (NFAT) is a family of transcription factors that affects cell survival, proliferation and

differentiation. NFAT plays key roles during development, including stimulation of axonal growth in neurons, maturation of immune system cells, heart valve formation, skeletal muscle and bone differentiation. Interestingly, NFAT can also differentiate immature cells in adults and promote tissue regeneration. In consequence, our goal was to evaluate the expression of NFAT in NPCs, investigating its possible role in NPC survival, proliferation and differentiation. Our findings indicate that NFAT is active not only in brain tissue from NFAT-luciferase reporter mice, but also in NPCs in culture. Moreover inhibition of NFAT activity in NPC cultures by a cell-permeable form of the highly-specific peptide VIVIT reduced neurosphere size and cell density in NPC cultures by decreasing proliferation and increasing cell death. NFAT inhibition also slowed NPC migration and astrocyte differentiation. In addition we identified NFATc3 as a NFAT isoform predominantly expressed in NPC cultures, finding that adenoviral expression of a constitutively-active form of NFATc3 inhibits NPC proliferation without increasing cell death, promotes NPC migration away from neurospheres and stimulates NPC differentiation into astrocytes and neurons. In summary, our work uncovers the active role of NFAT in NPC survival and differentiation, links NFAT to brain development and highlights its therapeutic potential for tissue regeneration.

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T11-07A

The ependymal region of the adult human spinal cord differs from other species and shows ependymoma-like features

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Question: Several laboratories have described the existence of undifferentiated precursor cells that may act like stem cells in the ependyma of the rodent spinal cord. However, there are reports showing that this region is occluded and disassembled in humans after the second decade of life.

Methods: To gain insight into the patency, actual structure, and molecular properties of the adult human spinal cord ependymal region, we used MRI on healthy volunteers and spinal cord injured patients. We also performed histology and laser capture microdissection followed by gene expression assays with human samples.

Results: We observed that the central canal is absent from the vast majority of individuals beyond the age of 18 years, gender-independently, throughout the entire length of the spinal cord, both in healthy controls and after injury. In addition, histology showed that morphology of the non-lesioned ependymal region is different from that described in other species, and revealed the presence of perivascular pseudorosettes, a common feature of ependymoma. With laser capture microdissection, followed by Taqman Low Density Arrays, we found that it adult human ependymal region is mainly enriched in genes compatible with a low grade or quiescent ependymoma, whereas show enrichment in a limited amount of genes related to neurogenic niches.

Conclusions: The presented data suggest that the ependymal region in adult humans is likely to be a reminiscent of a low-grade ependymoma, and a direct translation of the therapeutic potential of this region described in animal models to adult humans should be approached with caution.

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T11-09A

***Foxg1* antagonizes cortico-cerebral astrogenesis**

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Foxg1 is a transcription factor gene involved in key steps of early cortico-cerebral development, including specification of the telencephalic and cortical fields, tuning of proliferation/differentiation kinetics, radial migration of projection neurons and laminar specification of them. Its allele dosage is crucial. Hemizygosity for *Foxg1* and duplication of it result into two devastating nosological entities, namely the Rett and West syndromes, respectively. We previously showed that *Foxg1*, like its *Drosophila m.*ortholog *sloppy paired*, also antagonizes gliogenesis.

Aim of this study was to investigate the role played by this gene in two aspects of cortico-cerebral astroglial development, namely early commitment of neural stem cells towards glial fates and subsequent implementation of the astrocytic differentiation program. These issues were addressed in mouse and human models. In the first case, we took advantage of *Foxg1*-gain- and *Foxg1*-loss-of-function transgenic animals, in vivo electroporated brains and primary cultures of cortical precursors engineered by lentiviral vectors. As for human models, we relied on embryonic pallial precursors manipulated by lentiviral vectors and SFEBq aggregates derived from induced pluripotent stem cells.

It turned out that *Foxg1* overexpression not only antagonizes early glial commitment, but also interferes with selected aspects of late astrocytic differentiation. These findings may help to reconstruct the molecular logic underlying normal articulation of astrogenesis. Moreover they provide useful hints about pathogenetic mechanisms leading to neurological disorders triggered by altered *Foxg1* dosage.

T11-10A

Neurotransmitter and neurotrophin receptor expression by human dental pulp stem cells: implications for neural differentiation

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Dental pulp stem cells (DPSC) are originated from the neural crest, and believed to be directly derived from peripheral nerve system glia progenitors. Human DPSCs may constitute a privileged source of cells for their use in regenerative cell therapies, owing to their easy extraction, high rates of proliferation, and great differentiation versatility. Interestingly, although their predominant phenotype has been traditionally regarded to be mesenchymal, we demonstrate that DPSCs also retain a marked neural phenotype, which includes expression of early neural stem cell markers, peripheral nerve cell markers, neurotransmitter receptors, including functional ATP and kainate receptors, and even functional Voltage-dependent Na^+ and Ca^{2+} channels. Another very interesting property of DPSCs is that they express high amounts of neurotrophins and neurotrophin receptors. Importantly, both neurotransmitter and neurothrophin receptor expression levels change when cells are switched from fetal bovine serum-containing to serum-devoid culture medium. In particular, all high affinity neurotrophin receptors (TrkA, TrkB, and TrkC) increase greatly their expression when DPSC are cultured in serum-devoid medium, a fact which could be related to their neural differentiation. Consistently, when NGF, BDNF and NT3 are added to the culture medium, there occurs a drastic change in DPSC morphology, along with an up-regulation of some neural cell markers, down-regulation of connective tissue markers, and an activation of molecular switches involved in neurite growth and extension. Our results suggest that the expression of neurotransmitter and neurotrophin receptors by DPSC could be exploited to devise natural protocols that favor their differentiation to neural lineages, taking advantage of the intrinsic ability of these cells to undergo neural differentiation.

T11-11A

Aged neural stem cells in the hippocampus

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A population of radial neural stem cells (rNSCs) located in the subgranular zone of the dentate gyrus generates new neurons in the hippocampus throughout adulthood. These rNSCs share properties with astrocytes, have restricted mitotic potential and their number decline with age through conversion into astrocytes. We are now investigating the properties of the aged dentate gyrus, with a special focus on rNSCs using transgenic mice. Our results show that rNSCs undergo morphological changes such as increased number of primary ramifications, retraction of the apical arborization, thickening of the processes and increased expression of markers such as nestin and GFAP. The number of contacts with neurons, as well as the manner of distribution of rNSCs along the SGZ, also changes with age. These changes might be potentially affecting the way rNSCs respond to stimuli and neuronal activity. To test this hypothesis we will also analyze re-entry in the cell cycle as a measure of rNSC activation. These results will shed light into the process of aging of the hippocampus.

T11-12A

The HS-modifying enzyme Sulf2 controls generation of a novel glial precursor cell sub-type in the ventral spinal cord

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In the vertebrate central nervous system, glial cells display highly distinctive morphological features and functional properties. Although recognized for their crucial functions in neural physiology, progress in characterizing the heterogeneity of glial cells has been hindered for years due to a limited repertoire of lineage-specific genes. In particular, it is still not known whether glial cells share the same degree of heterogeneity of their neuronal companions. Based on recent progress in unraveling generation of glial cells in the embryo, studying mechanisms that control normal glia cell development emerges as a promising way to get insights on glia cell heterogeneity. Faced with the need to obtain additional lineage-specific molecular markers, we previously developed a molecular screen aimed at identifying genes up-regulated at initiation of gliogenesis in the embryonic spinal cord. This screen allowed the identification of Sulf2, an extracellular heparan-sulfate modifying enzyme involved in regulating critical signaling pathways. We recently started investigating the role of Sulf2 in the control of gliogenesis and found a quite unanticipated function for this enzyme. We indeed showed that Sulf2 depletion in mouse, while not affecting production of oligodendrocyte precursor cells (OPCs) from Olig2 progenitors of the ventral spinal cord, impairs generation of only a subset of glial precursor cells, also originating from Olig2 progenitors but with a slight delay. We found that this discrete Olig2-positive cell population, in contrast to OPCs, does not up-regulate Sox10 but instead, express Sox2 and Nkx6.1 as they leave the progenitor zone. Again contrasting with OPCs that rapidly disperse throughout the gray and white matter, these cells remain located in the ventral gray matter, at least until perinatal stages. Together, our data indicate that, in the ventral spinal cord, two distinct populations of Olig2-expressing glial precursor cells are generated at different time windows and that Sulf2 plays a specific role in controlling production of only a subset of these progenitors. The exact identity of these cells and molecular mechanisms underlying the function of Sulf2 in controlling their generation are currently under investigation.

T11-13A

Region-specific differences in astrocyte plasticity in the mouse forebrain

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The adult mammalian forebrain contains different subtypes of astroglial cells including neural stem cells (NSCs). While during development astrocytes play a major role in homeostatic balance, guidance and support of neuronal migration, at adult stages they are involved in maintenance of the neural microenvironment. Besides the fact that astrocytes from different parenchymal forebrain regions show different morphological and ultra-structural features, it is still unknown to which extent specific astrocyte subtypes would be dedicated to specific functions. Previously, we compared data sets of transcriptome analysis of astrocytes from different regions of the adult intact forebrain and NSCs from the subependymal zone (Beckervordersandforth et al., 2010). Surprisingly, not only we revealed region-specific differences between diencephalic and cortical astrocytes at the transcriptional level but also showed that diencephalic astrocytes share a significant amount of their transcriptome profile with adult NSCs. To further investigate this striking finding at the functional level, we analyzed the capacity of cells from the diencephalon to form multipotent and self-renewing neurospheres - the most common *in vitro* read-out for stem cell properties. While cells from the adult cerebral cortex generated very few - if any- neurospheres, cells isolated from the diencephalon (sparing the region around the third ventricle) had a profoundly higher neurosphere forming capacity. We further showed that these diencephalic spheres are able to self-renew over several passages and have the capacity to generate neurons, astrocytes and oligodendrocytes when exposed to differentiation conditions. Importantly, genetic fate-mapping using the GLAST::Cre^{ERT2} mouse line confirmed that the neurosphere-forming cells indeed derived from astrocytes. These results suggest that a fraction of diencephalic astrocytes are endowed with neural stem cell potential *in vitro*, thereby revealing a novel parenchymal stem cell niche in the adult murine brain under physiological conditions.

Beckervordersandforth R., Tripathi P., Ninkovic J., Bayam E., Lepier A., Stempfhuber B., Kirchhoff F., Hirrlinger J., Haslinger A., Chichung L. D., Beckers J., Yoder B., Irmler M. and Götz M. (2010) In vivo fate mapping and expression analysis reveals unique molecular hallmarks of prospectively isolated adult neural stem cells. Cell Stem Cell 7, 744-58.

T11-14A

Do umbilical cord stem cells direct neural progenitor cells towards an oligodendroglial fate through paracrine factors or cell-to-cell contact?

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Question: Neonatal hypoxic-ischemic brain injury remains an important cause for long-term neurological deficits. Wharton's jelly mesenchymal stem cells (WJ-MSC) from the umbilical cord might be ideal candidates to cure perinatal brain damage. Their secretome has been shown in vitro and in vivo to have beneficial effects on neuroregeneration. Still, it is not clear if cell-to-cell contact may equally contribute to this positive effect. Therefore, the objective of this study is to elucidate through which of these two mechanisms neuroregeneration ought to be triggered the most in vitro.

Methods: The effect of WJ-MSC on the expression of neuroglial markers in neural progenitor cells (NPC) was assessed in vitro in conditioned medium and co-culture experiments by immunocytochemistry, real-time PCR and western blot. Furthermore, the differences between WJ-MSC derived from term or preterm deliveries were evaluated. Additionally the secretome of WJ-MSC was analyzed by mass spectroscopy and with a membrane-based antibody array.

Results: Hippocampal NPC at passage 3 showed an increased expression of glial markers such as myelin basic protein (Mbp), galactocerebroside (GalC) or glial fibrillary acidic protein (Gfap) after exposure to WJ-MSC-conditioned medium (CM) or after direct contact to WJ-MSC. Interestingly, WJ-MSC from term deliveries induced more strongly the expression of glial markers when compared to preterm. Co-cultures with direct cell-to-cell contact had a more prominent effect on the expression of glial markers compared to CM or transwell co-cultures.

Conclusions: WJ-MSC derived from term deliveries have a different secretome compared to preterm births. This was demonstrated by mass spectroscopy as well as by in vitro experiments. Moreover, cell-to-cell contact may be decisive to induce oligodendroglial differentiation on resident NPC. In

conclusion, transplanting WJ-MSC into damaged brains of neonatal infants may enhance and support endogenous remyelination and neuroregeneration.

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T11-15A

Compensatory mechanisms in the age-induced decline of adult hippocampal neurogenesis

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Neurogenesis, the process of generating functional new neurons, persists in the subgranular zone (SGZ) of the hippocampal dentate gyrus throughout life. During aging, the SGZ neurogenic capacity undergoes a progressive decline, which can mainly be attributed to loss neural stem cells. However, the remaining steps of the neurogenic cascade, namely stem and progenitor cell and proliferation, and neuronal survival and differentiation are also altered during aging to potentially compensate for the loss of neural stem cells. In this study, we compared the SGZ niche of 1, 2, 6 and 12 month-old mice to assess all phases of adult hippocampal neurogenesis. In agreement with previous reports, we observed a dramatic decline in SGZ proliferation associated with age, but no major alterations in survival and differentiation rates. Our data suggest that the abrupt age-related neuronal loss is not counteracted by any compensatory mechanism, neither of stem cell proliferation, nor newborn cell differentiation and survival. Therefore, the age-related decline in neurogenesis is largely explained by the loss of neural stem cells alone.

T11-16A

Towards mobilizing the brain's own neural stem cells to restore striatal dysfunction in Parkinson patients

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Neural stem cells (NSCs) shape the brain during development by differentiating into different specialized brain cell types including neurons, astrocytes and oligodendrocytes. It is now widely accepted that new neurons are born throughout life. The subventricular zone (SVZ) and the dentate gyrus of the hippocampus are considered to be the classical neurogenic regions in the adult human brain. It is this characteristic that holds a promise for future therapy for neurodegenerative disorders such as Parkinson's disease (PD). In PD, striatal depletion of dopamine is underlying the movement problems. Since the striatum is neighboring the SVZ, NSCs that are present in the SVZ are an attractive target to develop future PD therapies based on activating the brain's own repair capacity. Our group showed that NSCs are still present in the brains of PD patients and that these cells are still able to proliferate and differentiate *in vitro.* However, a prerequisite for therapy based on endogenous NSCs is to know more about the characteristics of these NSCs in the elderly and diseased brain.

The hypothesis is that the SVZ NSCs express specific receptors that can be stimulated by growth factors in the CSF to increase neurogenesis leading to repair of the striatal dysfunction.

To study this, we have established a technique to specifically isolate and culture adult human NSCs from the post-mortem human SVZ of elderly donors. These cells formed neurospheres and were able to differentiate into neurons, astrocytes and oligodendrocytes. We use this unique method to isolate NSCs from the SVZ of elderly control donors and PD patients and we will compare these cells based on proliferation and differentiation models and microarray and we will create cell-lines.

Mobilizing the endogenous SVZ NSCs to replenish striatal dopamine is an attractive approach to alleviate the motor symptoms in PD patients. Our novel technique to isolate and culture these cells from the patient's brain will allow, for the first time, extensive molecular and cellular analysis of these

cells, which is essential to develop future therapies based on activating the brain's own repair capacity.

T11-01B

DNA methylation in ageing adult oligodendrocyte progenitor cells

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Question: The efficiency of CNS remyelination decreases with ageing. Aberrant epigenetic patterns (such as DNA methylation, histone modifications and microRNAs) accumulate with age and contribute to the ageing phenotype. Epigenetic mechanisms such as histone acetylation are involved in the differentiation of oligodendrocyte progenitor cells (OPCs) into mature myelinating oligodendrocytes. Moreover, declining histone acetylation activity during the process of ageing leads to a decreased efficiency of adult OPC differentiation.

In this study we further explore the underlying epigenetic layers that are involved in the control of differentiation of OPCs, focusing specifically on the effect of DNA methylation in OPC in the young and aged adult brain.

Methods and results: Using isolated adult rat OPC, we investigate the role of DNA methylation in OPC differentiation *in vitro*. We use DNA methyltransferase (DNMT) inhibitor in order to show that inhibition of DNA methylation reduces differentiation of adult OPC *in vitro*. Furthermore, comparing young and aged OPC cell cultures we show a dynamic expression of the DNMT enzymes during OPC differentiation *in vitro*. In addition, we have also characterized DNA methylation in ageing OPCs using various genomic analyses.

Conclusions: Our study supports a model in which aberrant DNA methylation in ageing OPC may play a crucial role in the age-associated decreased efficiency in remyelination.

T11-02B

The impact of TNFα on the developing brain

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Maternal immune activation (MIA) is known to affect neuronal precursor gene expression, proliferation and differentiation, and has been shown in mice to produce abnormal phenotypes resembling human neurodevelopmental disorders, particularly schizophrenia and autism. The relative contributions of the maternal and foetal immune response is unknown, and the precise role of the microglial cell in normal and pathological brain development has yet to be fully elucidated. To investigate the contribution of TNFα, we bred TNFα heterozygous C57BL6 mice to generate offspring with 5 different combinations of maternal and foetal TNFα gene dosage. These 5 groups were treated with either saline or LPS (10μg/kg) at E13 and killed at E13.5. Normal saline-injected C57BL6 mice were used as controls. Phosphohistone-H3 positive mitotic cells were counted in the embryonic proliferative zones (ventricular and subventricular zones of the dorsal and medial cortex) at E13.5, and microglial number and arborisation was assessed by Iba1 immunohistochemistry. Counts of proliferating cells were corrected for variation in the size of the proliferative zones. In the SVZ of the dorsal cortex, the density of proliferating cells was increased by maternal TNFα knockout in a gene dosage-dependent manner. In the VZ of the dorsal cortex, both heterozygous and knockout foetuses showed decreased density of mitotic cells, with no effect of maternal genotype. Immature microglia were found sparsely populating

the embryonic brain at E13.5, with both heterozygous and knockout foetuses showing decreased numbers of microglia and an increase in the fraction of non-arborised (rounded) microglia. In the dorsal and medial SVZ, LPS caused a decrease in mitotic cell density. In the dorsal SVZ, density of proliferating cells was much lower in foetuses from KO and Het mothers, while foetal KO status exacerbated the effect of maternal KO status. Together, our results show differential sensitivity to both maternal and foetal TNFα status in different proliferative zones of the brain, with pro-proliferative non-linear effects of foetal TNFα status in the dorsal VZ correlating with increased microglia numbers and arborisation and linearly anti-proliferative effects of maternal TNFα status in the dorsal SVZ. Thus, our research shows that microglial cell number is dependent on foetal TNFα status, but that maternal TNFα signalling can directly or indirectly have a profound effect on the development of the foetal brain independently of the ability of the foetus to produce TNFα.

T11-03B

Study of the capability of endogenous neural stem cells to protect from glutamatergic excitotoxicity by sensing danger signals

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In the adult rodent brain, neurogenesis occurs mainly in two regions: the subventricular zone (SVZ) of the lateral ventricles and the subgranular zone of the dentate gyrus. Adult endogenous neural precursor cells (eNPCs) in the SVZ are known to contribute to long-term neurogenesis in the olfactory bulb, moreover the concept that SVZ eNPCs might sense danger signals triggering a protective response is emerging. We have previously shown that SVZ eNPCs are able to protect striatal neurons from glutamatergic excitotoxicity by releasing endocannabinoids (Butti et al., 2012). Toll-like receptor 4 (TLR4) is triggered by several types of endogenous and exogenous danger signals, including those released after glutamate-induced excitoxicity occurring in the early phases of ischemic stroke.

Aim of this study was to investigate whether eNPCs possess danger signal receptors able to trigger the secretion of neuroprotective cues.

We observed the expression of TLR4 in cultured eNPCs of the SVZ derived from healthy and stroke-affected mice. To distinguish which type of neural stem cells (B, C or A) express this receptor we performed cytofluorimetric analysis using stem markers, such as prominin-1, EGFR and nestin of ex vivo healthy SVZ and our preliminary results suggest B cells having the higher expression of TLR4. Moreover, we performed the characterization of the diverse NPC populations in wild-type and $TLR4^{-/-}$ mice in terms of clonogenic assay, growth rate and differentiation potential and we found that $TLR4^{-/-}$ NPCs show reduced cell proliferation and increased differentiation capacities. Also in vivo, we observed that the lack of TLR4 induces an inhibition of neurogenesis. These results suggest an important role for TLR4 to maintain eNPCs. The next step will be then to investigate the role of TLR4 in mice with ischemic stroke using a specific eNPCS TLR4 deficient transgenic mice.

T11-04B

Enteric Glia: S100b, GFAP and beyond

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The enteric nervous system (ENS) harbours neurons, glial cells and their precursors derived from the neural crest. In recent years, the key role of enteric glia in supplying neurotrophic support, responding to inflammation or being the source for neural stem cells has become increasingly obvious. Currently more glial subtypes are proposed to fulfil the different tasks.

Based on several transgenic models (Nestin-GFP, hGFAP-EGFP and PLP1/DsRed) histological studies were performed to deliver a basic inventory which includes general (S100b), and reactive (GFAP) glial, as well as neural stem cell marker for the different parts of the gastrointestinal tract (GIT) in postnatal and adult mice under homeostatic conditions.

We detected nestin-expressing cells which mainly express the glia markers S100b and GFAP throughout the GIT from the stomach to the distal part of the colon at the postnatal and adult stages. A nestin expressing neuronal population is present in the myenteric plexus which was decreased from the postnatal to adult stage but with an increased expression of neuronal markers at the adult stage in the caecum.

Intriguingly, $S100b^{+}$ $GFAP^{+/-}$ $Sox2^{-}Sox10^{-}$ $Nestin^{-}HuC/D^{+}$ $PGP9.5^{+}$ $PLP\text{-}1^{-}$ cells with a neuronal morphology were mainly located in the jejunum in postnatal mice. Compared to the postnatal stage these cells were rarely found in the intestine of adult mice. PLP-1+ enteric glial cells were found with different morphologies in the GIT.

In conclusion, the presence of premature neurons that express nestin and glia-neuron intermediate cells demonstrates that neurogenesis takes part far beyond birth. Moreover, beside S100b and GFAP expressing enteric glial cells, there is an additional subset of glia that expresses the Schwann cell marker PLP-1.

T11-05B

Molecular and cellular characterization of the dormant and injury- activated mouse and human spinal cord stem cell niches

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The adult spinal cord contains a pool of neural stem cells in a niche situated around the central canal. These cells, which are present in rodents and in man, are able to form new neuronal and glial cells in vitro. In contrast to the brain where neural stem cells are actively engaged in adult neurogenesis, the spinal cord niche is in a dormant state and produces no or few new cells in the normal situation. However, the niche can be readily activated and generate new cells in spinal cord traumas or spinal cord degenerative pathologies such as amyotrophic lateral sclerosis.

Over the last few years, our lab has characterized the human and mouse spinal cord niches in depth. We showed that far from being a single layer of cells, the central canal region is composed of several cell types which show immaturity features and that the niche maintains the activation of several developmental signalling pathways.

Recently, we explored the molecular mechanisms underlying the activation of the niche after injury. We used laser microdissection combined with microarray screening to decipher the early molecular events taking place during activation. This led us to show that the Mapk/Ras signalling is rapidly activated in the niche after injury and we identified several potential targets of this pathway which may orchestrate the activation. In order to describe further the spatial organisation of the niche we microdissected the ventral, lateral and dorsal parts of the central canal. This will allow us to identify genes which are more specific of the different regions of the niche. To explore the conservation of the niche in mammals, the human spinal cord niche is also explored by immunohistochemistry and transcriptome analysis.

This work will generate a new body of knowledge on the spinal cord niche which can provide some essential data to understand and may be control the activation and the differentiation of spinal cord stem cells. In depth, characterization of the spinal cord niche constitutes a framework to make the most of this endogenous cell pool useful in treatment of spinal cord diseases.

T11-06B

Mining the sorting machinery of extracellular miRNAs in neural stem/precursor cells

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Introduction: Neural stem/precursor cell (NPC) transplantation protects the central nervous system from inflammatory damage via cell-to-cell communication mechanisms. Recent works suggest that the exosome-mediated transfer of molecules such as microRNAs (miRNAs) might play an important role in mediating the protective effect of NPCs. Here we aim to identify the machinery that sorts miRNAs to exosomes in murine NPCs. Our hypothesis is that such a mechanism might act 1) at the transcriptional level, with transcription factors (TFs) driving the transcription of exosomal miRNAs; or 2) at the post-transcriptional level, with carrier proteins that recognize specific miRNAs, bind to them and mediate their export to exosomes.

Methods: We used RNA-Seq to identify miRNAs significantly more abundant in exosomes than parental cells. To address whether specific TFs drive the transcription of secreted miRNAs, we tested whether any TF binding site is enriched in their promoters. In parallel, we used a variety of motif enrichment tools available in R/Bioconductor (Cosmo, BCRANK, motifRG) to find short motifs enriched in secreted miRNAs.

Results: We found that no specific TF binding site is enriched in the promoters of secreted miRNAs. However, we identified two short motifs over-represented in exosomal miRNAs, one of which matches the binding sequence of hnRNPA2B1, which previous works have shown to be involved in miRNA secretion. By western blot we found that hnRNPA2B1 is not present within NPC-derived exosomes, suggesting that other proteins might be involved in miRNA secretion in NPCs.

Conclusion: Exosomal miRNAs do not seem to be regulated by specific TFs. We are currently investigating whether other proteins and/or other miRNA features (such as the pre-miRNA secondary structure) might be responsible for miRNA secretion in NPCs. Altogether, this work will help to shed light on the molecular mechanism behind miRNA trafficking and on its implication on the therapeutic effect of transplanted NPCs.

T11-07B

Mesenchymal stem cell-secreted factors prevent $p57^{kip2}$ nuclear translocation in neural stem/progenitor cells: role in oligodendroglial fate decision?

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Question: The generation of new oligodendrocytes and the repair of myelin sheaths represent processes that are essential for enhancing the ability of the brain to repair itself. These so-called remyelination phases follow the damage caused by diseases like multiple sclerosis in the adult central nervous system (CNS). We identified the multifunctional $p57^{kip2}$ protein as a negative regulator of myelinating glial cell differentiation and as an important intrinsic switch for glial fate decision in adult neural stem/progenitor cells (aNSPCs) (Jadasz et al., 2012). Furthermore, others and we have also shown that external stimulation of aNSPCs with mesenchymal stem cell (MSC)-secreted factors enhances oligodendrogenesis by yet unknown mechanisms (Jadasz et al., 2013). Published data suggest that a translocation of the $p57^{kip2}$ protein in oligodendroglial progenitor cells promotes differentiation (Göttle et al., 2015). Here, we address the question whether the MSC-derived oligodendrogenic effect on aNSPCs involves a translocation mechanism of the glial fate regulator $p57^{kip2}$.

Methods: To this end we analyzed the localization of the $p57^{kip2}$ protein within aNSPCs after stimulation with MSC-conditioned medium (MSC-CM) from rat and human sources and in addition under astroglial differentiation stimuli. Moreover, we also analyzed the expression of prominent oligodendroglial and astroglial markers.

Results: The exogenous stimulation of aNSPCs with MSC-CM resulted in increased cytoplasmic localization of p57kip2 whereas after astroglial stimulation the percentage of nucleic $p57^{kip2}$ localization was significantly enhanced. Interestingly human MSC-CM resulted in similar translocation effects and contributed to oligodendrogenesis of aNSPCs.

Conclusions: We thus conclude that MSC-secreted factors prevent $p57^{kip2}$ nuclear translocation in aNSPCs and that this event might be involved in the oligodendrogenic molecular mechanism induced by MSCs.

T11-09B

Neural stem cell therapy for spinal cord injury

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Spinal cord injury (SCI) causes partial or total loss of motor, autonomic and sensory functions below the level of the lesion mainly due to interruption of spinal pathways and secondary neurodegenerative processes. The transplant of Neural Stem Cells (NSCs) is a promising approach, since NSCs are able to differentiate and integrate within the damaged tissue, inducing regeneration of the neuronal network. Reprogramming of adult somatic cells into induced Pluripotent Stem Cells (iPSCs) is expected to provide an autologous source of human induced NSCs (hiNSCs), avoiding the ethical issues concerning the use of stem cells from embrionic and fetal tissue. Although the characterization of hiNSCs transplanted after SCI has been already studied, there is still limited information on the behaviour and differentiation pattern of the transplanted hiNSCs within the damaged tissue. Thus, we transplanted rats with hiNSCs at 0 days and 7 days after SCI. We histologically analyzed engrafment, proliferation and differentiation of the hiNSCs and the spared tissue in the spinal cords at 7, 21 and 63 days post-transplant. We also evaluated locomotor function of the animals during the follow-up. Both groups showed a clear deficit of functional locomotion compared to vehicle-injected groups. Histological analysis showed high proliferation of the transplanted cells within the tissue, forming a cell mass that may explain the decline of motor function with time. Most of the hiNSCs differentiated into neural and astroglial lineages, whereas no differentiation into oligodendrocytes was observed. Furthermore, some cells remained still undifferentiated and proliferating at the final time, resulting in uncontrolled expansion of the cells within the injured tissue.

T11-10B

Neuroinflammation influences the viability, distribution and therapeutic efficacy of transplanted neural stem cells in a mouse model of multiple sclerosis

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Introduction: Intrathecal transplantation of adult neural stem/precursor cells (NPCs) ameliorates disease severity in experimental autoimmune encephalomyelitis (EAE), the animal model of multiple sclerosis. However, the kinetics of NPC survival and localization after intrathecal transplantation and the effect of neuroinflammation on NPC behaviour and therapeutic efficacy remain largely unknown. **Methods**: NPCs were derived from the subventricular zone of 8-week old female C57Bl/6 mice. EAE was induced in syngeneic mice by subcutaneous immunization with myelin oligodendrocyte glycoprotein 35-55 peptide. One million GFP-labelled NPCs were transplanted intrathecally in the cisterna magna of EAE mice at peak of disease severity or healthy matched controls (HC). NPC survival and localization was assessed by immunohistochemistry at 1, 7 and 60 days post transplantation (dpt). Each group comprised at least 4 animals. **Results**: At 1 dpt, NPCs distributed within few millimetres from the injection site (2,46 ±0,90 mm in EAE; 2,78 ± 0,57 in HC), no further migration was observed at 7 and 60 dpt. At 1 dpt, 8,1% of transplanted NPCs survived in HC and 7,5% in EAE. At 7 dpt the number of surviving NPCs further decreased in both groups (HC: 2,6%; EAE: 4,6%). Indeed, a fraction of transplanted NPCs expressed the apoptotic marker activated

caspase 3, with EAE mice showing a trend of reduced apoptosis at 1 dpt (HC: 3,2%; EAE: 1,5%) and at 7 dpt (HC: 3,8%; EAE: 1,4%; p<0,05). Consistently, at 60 dpt NPCs transplanted in EAE mice displayed increased survival (2,7%) when compared to HC (0,3%; p<0,05). In both groups, transplanted NPCs localized mainly in the subarachnoid spaces of the fourth ventricle or surrounding meninges at 1 dpt (EAE: 94,8% of surviving NPCs; HC: 87,6%) and 7 dpt (EAE: 98,5%; HC: 89,4%), with a small quota of NPCs integrating in the parenchyma. At 60 dpt, 93,5 % of surviving NPCs retained their meningeal localization in the EAE group, while in the HC group 89,4% of the surviving NPCs were found in the parenchyma. Importantly, therapeutic transplantation of NPCs in the chronic phase of EAE (80 dpi), when neuroinflammation has waned off, failed to induce clinical amelioration when compared to transplantation of NPCs in the acute phase of EAE. **Conclusions**: The inflammatory environment of EAE does not affect NPC survival in the immediate post-transplant phase. However, neuroinflammation might influence long-term the viability, survival and therapeutic efficacy of transplanted NPCs in EAE.

T11-11B

Neurogenesis and lateral ventriclar extension in the adult guinea pig brain

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Neuroblasts generated in the subventricular zone (SVZ), migrate to the olfactory bulb (OB) through a pathway called the rostral migratory stream (RMS). Although the RMS is not present in the human brain, a migratory pathway organized around a ventricular cavity (extension of the lateral ventricles, eLV) that reaches the OB has been reported. A similar eLV structure is found in the guinea pig brain; however, neurogenesis and precursor cell migration has not been described in this species. Therefore, we have analyzed the neurogenic activity and precursor cell migration in the SVZ and eLV of the adult guinea pig brain. We analyzed the SVZ, eLV and OB in 1, 6 and 12 month-old guinea pig brains. We performed bromodeoxyuridine (BrdU) labeling to analyze proliferation. Immunohistochemical analysis, confocal microscopy and electron microscopy were used to study the cytoarchitecture of the guinea pig SVZ and eLV. We identified neuroblasts (A cells, bIII tubulin +), precursors cells (B and C cells, BrdU +), and ependymal cells (E cells, vimentin +) in the SVZ and eLV. The eLV was lined by ependymal cells and was surrounded by migrating neuroblasts. The average number of neuroblasts per section increased progressively from the SVZ to the BO, reaching 2559 ± 164.5 neuroblasts in the OB. Ultra-structural analysis confirmed our results; the ependymal cells have cilia and microvilli on their apical surface, and neuroblasts and astrocytes were densely packed under the ependymal wall. After 24 h, BrdU labeling revealed that BrdU + cells were mainly located in the SVZ, with few BrdU + cells in the eLV and OB. In addition, the average number of BrdU + cells decreased in the SVZ and increased in the OB 1, 5 and 10 days after BrdU labeling, indicating that neuroblasts migrate from the SVZ to the OB. Finally, analysis of neurogenesis in 1, 6 and 12 month-old guinea pigs revealed that the eLV structure was preserved in older animals; however, the average number of neuroblasts and BrdU + cells in the SVZ and eLV decreased progressively in older animals. In the adult guinea pig brain, the eLV connects the LV with the OB in a fashion similar to that described in the human brain. A stream of migrating neuroblasts is organized around the eVL migration path that has no neurogenic potential. In older guinea pigs, neurogenesis decreases in a similar way to that described in other species. Therefore, we propose that the guinea pig brain may be used as a new neurogenic model with closest similarity to that observed in humans.

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T11-12B

Myelinating oligodendrocytes generated by direct cell reprogramming from adult rat adipose tissue

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Obtaining oligodendroglial cells from dispensable tissues would be of great interest for autologous or immunocompatible cell replacement in demyelinating diseases as well as for studying myelin pathologies. Recently, two laboratories have simultaneously reported that mouse fibroblasts could be converted into oligodendroglial cells by direct reprogramming with transcription factors involved in oligodendrocyte development (Najm et al., Nat. Biotechnol. 31:426, 2013, Yang et al., Nat. Biotechnol. 31:434, 2013). We have lentivirally transfected combinations of tetracycline-inducible sox10 (S), olig2 (O), zfp536 (Z) and/or nkx6.1 (N) transgenes in adult rat adipose tissue-derived stromal cells (ADSCs) and checked for the generation of functional oligodendrocytes. Immunostaining with the O4 monoclonal, which binds to oligodendrocyte progenitor cell membranes, was used as initial marker of oligodendroglial reprogramming. The first signs of ADSC conversion into oligodendrocytic cells were observed with the S-O-Z combination by 6 weeks and the O4+ cell population kept rising the following months, eventually constituting more than 50% of cells. Increasing numbers of O1 (galactocerebroside), p75 (low-affinity NGF receptor), and GFAP-positive cells appeared in that period. Cells expressed also myelin-associated proteins like MBP, MAG, MOG and PLP1/DM20. Transduction with the S-O-N-Z transgene combination also produced oligodendrocyte progenitors but did not improve the result; S-O-N, O-Z or O transductions did not consistently produce oligodendrocytes in our hands. After 3 months of continuous expansion, the reprogrammed cells became independent of transgene activation by doxycycline. Antibiotic selection of transduced cells (with zeocin) did not show to be more efficient for reprogrammed cell purity and proliferation than unselected cultures. Different pre-induction treatments were tried in an attempt to improve the efficiency or speed of reprogramming: of these, treatment with Repsox followed by retinoic acid or adipocytic pre-differentiation, appeared to increase reprogramming consistency or to slightly accelerate the process. When seeded onto rat dorsal root ganglion neurons, transdifferentiated cells were observed to cover lengths of one or several axons with typical myelin-like appearance. We propose that functional oligodendrocytes can be efficiently generated from adult mesenchymal cells in the rat by direct cell reprogramming.

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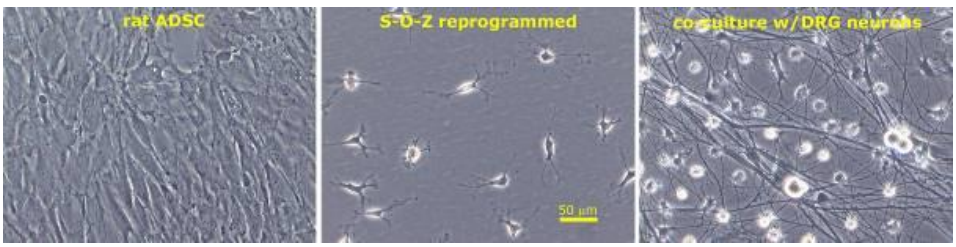
Image



T11-13B

Fate potential and clonal analysis of neural progenitors in distinct germinal niches of the postnatal cerebellum

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The cerebellum comprises a wide variety of neuronal and glial phenotypes that all derive from two distinct embryonic neuroepithelia: the ventricular zone (VZ) and the rombic lip. From the first one progenitors with astroglial traits delaminate to the cerebellar parenchyma where they establish secondary proliferative niches active during the early postnatal life: the Purkinje cell layer (PCL)

populated by radial Bergmann glia precursors and the prospective white matter (PWM) where immature interneurons become postmitotic. We firstly established that distinct proliferative machineries are active in these niches. These findings were in line with the different proliferative rates of those territories and suggested different differentiation potentials of the residing progenitor populations. Thus, we performed a genetic fate mapping study of GlastCreER^{T2}xR26R mice to understand the fate potential of these niches and the lineage relationships in their progeny. Data showed that some proliferating Glast^{+}-precursors possess a neurogenic capability after birth. To get further insight in their behaviour, we topically tagged PCL or PWM residing progenitors and we found that interneurons were exclusively generated by progenitors in the PWM. Conversely, PCL astroglial progenitors possess only gliogenic potential. Finally, *in vivo* clonal analysis of Glast^{+}-precursors in Confetti mice confirmed and further extended these findings indicating that PWM progenitors are bipotent and able to generate both interneurons and white matter astrocytes while PCL-progenitors produce Bergmann glia and granule cell layer astrocytes.

T11-14B

Low density lipoprotein receptor-related protein 1 (LRP1) - a novel modulator of neural stem cells' properties in the developing cortex and spinal cord

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The LDL family of receptors and it's member LRP1 has classically been associated with a modulation of lipoprotein metabolism. However, recent studies indicate a diversity of roles for these receptors in various aspects of cellular activities, including cell proliferation, migration, differentiation and survival.

LRP1 is essential for the normal neuronal function in the adult CNS, whereas the role of LRP1 in development remained unclear.

Previously we have observed a high upregulation of LewisX glycosilated LRP1 in the stem cells of the developing cortex and demonstrated it's importance for oligodendrocyte differentiation.

In the present study we show that LeX-glycosilated LRP1 is also expressed in the stem cell compartment of the developing spinal cord, and has broader functions in the developing CNS.

We have investigated the basic properties of LRP1 conditional knock-out NSPCs (neural stem precursor cells) from cortex and spinal cord, created by means of Cre-loxp mediated recombination in vitro. The functional status of LRP1-deficient cells has been studied using proliferation, differentiation and apoptosis assays.

LRP1 deficient NSPCs from both CNS regions demonstrated a modified differentiation profile. Their differentiation capacity towards OPCs, mature oligodendrocytes and neurons appeared reduced. In contrast, astrocytic differentiation was enhanced.

Moreover, LRP1 deletion had a negative effect on NSPCs proliferation and survival.

Our observations suggest that LRP1 facilitates NSPCs differentiation via interaction with APOE. Thus, upon APOE4 stimulation wt radial glia cells generated more oligodendrocytes and neurons, but LRP1 knock out cells showed no response. The response to APOE seems to be independent of cholesterol uptake or MAPK and Akt activation. Currently, further downstream mechanisms are being considered.

T11-15B

Characterization of neural stem cell-derived reactive astrocytes in LPAR1-EGFP mice

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Radial neural stem cells (rNSCs) persist in the hippocampus of most mammals and are able to generate neurons through adulthood, a process known as adult neurogenesis. Adult hippocampal neurogenesis is important for spatial memory, pattern separation and the responses to fear, stress and anxiety. rNSCs are mostly quiescent but once they are activated, they generate neuronal precursors through several rounds of asymmetric division and then differentiate into astrocytes, losing their stem cell capabilities. We have recently discovered that seizures originated in the hippocampus massively activate rNSCs and induce them to change their morphology, to enter symmetric cell division and to differentiate into reactive astrocytes (RAs). RAs are key players in the brain's response to injury because they are proinflammatory and disrupt synaptic transmission.

We aim to characterize rNSC-derived RAs and compare them with those RAs differentiated from parenchymal astrocytes in the hippocampus after seizures. We hypothesize that rNSC-derived RAs might be different form astrocyte-derived RAs and play a key role in the development of hippocampal reactive gliosis in response to seizures.

To distinguish rNSC-derived RAs from astrocyte-derived RAs we are employing the transgenic mouse line LPAR1-EGFP. Our results show that LPAR1-EGFP expression is highly restricted to rNSCs in the hippocampus. Furthermore, LPAR1-EGFP expression is maintained in rNSCs over a period of several weeks, as they differentiate into RAs due to seizures. On the contrary, astrocyte-derived RAs do not express LPAR1-EGFP at any time point. Thus, expression of LPAR1-EGFP is a unique tool to track the morphological and functional changes undergone by rNSCs to differentiate into RAs after seizures.

T11-16B

Efficient derivation of myelinating oligodendrocytes from NKX2.1-GFP human embryonic stem cell reporter line

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Transplantation of human embryonic stem cell (hESC)-derived oligodendrocyte precursors (OPCs) has been considered as a potential therapeutic strategy for acquired demyelinating, or inherited hypomyelinating disorders. However, the yield of homogeneous hESC-derived OPCs is a limiting factor of current protocols in attempt, to exclude any unwanted immature or other lineage cells that may form gliomas. To increase the homogeneity of hESC-derived OPCs, we utilised the NKX2.1-GFP hESC reporter line and sorted GFP+ and GFP- cells through fluorescent-activated cell sorting at the neural precursor stage of differentiation, based on their peak GFP expression. Subsequently, these two populations underwent further differentiation towards the oligodendroglial lineage under defined conditions. The differentiation potential towards the oligodendroglial lineage was compared between GFP+ and GFP- cell populations. Their oligodendroglial lineage commitment was determined at specific time points during each stage of differentiation by the presence of various antigens which included Nestin, PDGFRα, NG2, O4, MBP, GFAP, β-III tubulin, and Epcam by flow cytometry and immunocytochemistry (ICC). Furthermore, the expression of transcription factors that drive oligodendrogenesis such as Sox10, Olig2 and Nkx2.2 were demonstrated by ICC and qRT-PCR. Our data show that a high yield of homogeneous OPCs could be derived from the NKX2.1-GFP hESC reporter line subsequent to GFP-based sorting (60% of PDGFRα+/NG2+ OPCs from GFP+ populations compared to 2% of PDGFRα +/NG2+ OPCs from GFP- population). The yield of O4+ pre-myelinating oligodendrocytes was significantly higher in GFP+ populations (45%) compared to the GFP- population (<1%). Furthermore, these O4+ pre-myelinating oligodendrocytes from GFP+ populations were able to myelinate 30% of axons from rat retinal ganglion cells. Consequently, myelinating oligodendrocytes were efficiently derived from the NKX2.1-GFP hESC reporter line, suggesting a positive role for NKX2.1 during *in vitro* human oligodendrogenesis. Further studies based on these preliminary data may enhance the understanding of oligodendrocyte development and contribute to therapeutic mechanisms for patients with demyelinated or inherited hypomyelinating disorders.

Poster topic 12
Neuroimmunology and neuroinflammation

T12-01A

Intravenous immunoglobulin protects oligodendrocytes in an organotypic slice culture model for demyelination

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Intravenous immunoglobulin (IVIG) is a polyvalent mixture of purified immunoglobulins pooled from the plasma of healthy donors. While IVIG is a well-tolerated treatment for various autoimmune conditions, it is still under debate, whether IVIG is beneficial in the treatment of Multiple Sclerosis. We therefore aimed to elucidate the effects of IVIG on demyelination in an *in vitro* model of the CNS - immune interface.

Using organotypic slice cultures (OSC) from transgenic mice, which express GFP in oligodendrocytes, extensive demyelination and oligodendrocyte loss was induced over three days with an anti-myelin oligodendrocyte glycoprotein (MOG) antibody and complement.

In the model of immune-mediated demyelination, IVIG effectively preserved oligodendrocyte and myelin integrity - as documented by live imaging of GFP expression, confocal microscopy and gene expression analysis - in a dose dependent manner. Staining of living OSC with propidium iodide (PI) or anti-CD68 mAb confirmed that IVIG also prevented antibody/complement-induced cell death and microglial activation, respectively, as compared to untreated controls. In contrast to whole IVIG, neither a monoclonal humanized IgG antibody nor equimolar IVIG-derived Fab-fragments exerted a protective effect, while Fc-fragments from a human polyclonal IgG preparation was as efficient as whole IVIG.

In this *in vitro* model of the CNS-immune interface IVIG protected from immune-mediated CNS demyelination. Our data indicate that these IVIG effects rely partly on interfering with the complement cascade and on interaction with local immune cells.

T12-02A

Age-related changes in glial functionality in hippocampal astrocytes: the role of NFκB, p38, Nrf-2 and HO-1 pathways in inflammatory response

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Astrocytes, a major class of glial cells, are dynamic cells that maintain brain homeostasis, regulating neurotransmitter systems, synaptic information processing, energy metabolism, maintenance of the blood-brain barrier, antioxidant defenses and inflammatory response. Astrocytes are key cells in neuroinflammation, because they sense and amplify inflammatory signals from microglia and/or initiate inflammatory mediators release that are strictly related to transcriptional factors, such as nuclear factor erythroid-derived 2-like 2 (Nrf-2) and nuclear factor kappa B (NFκB). Additionally, aging is closely associated with changes in glial functionality and increase of inflammatory mediators. Astrocyte cultures derived from rodent brains have been extensively used to characterizeastrocytes' biochemical, pharmacological and morphological properties. Thus, this study introduces a routine protocol for hippocampal primary astrocyte cultures from adult (90 days old) and aged (180 days old) Wistar rats as tool for aging studies. Forthis we used enzymatic digestion (trypsin) and mechanical dissociation. Medium exchange occurred from 24 h after obtaining a culture and after, twice a week up to reach the confluence (around the 4^{th} to 5^{th}week). Under basal conditions, adult and aging astrocytes presented a polygonal to fusiform and flat morphology. We analyzed the classical glial proteins by

immunocytochemistry and Western blotting. Significant age-dependent changes in glial responses were found. Classical cytoskeleton proteins such as glial fibrillary acidic protein (GFAP) and vimentin as well as glutamate transporters (GLAST and GLT-1) were detected and changed their expressions with age. Measurement of proinflamamtory cytokines was carried out in an extracellular medium, using ELISA from commercial kits. Astrocytes displayed age-dependent inflammatory response with augment of proinflammatory cytokine levels such as TNF-α, IL-1β, IL-6, IL-18 and chemokine MCP-1. The putative mechanisms associated to age-dependent changes in glial functionality in hippocampal astrocyte cultures involve NFκB, p38, Nrf-2 and HO-1 pathways. These results reinforce the role of hippocampal astrocytes as target for understanding mechanisms involved in aging and age-related neurological disorders.

T12-03A

Translational investigation of microglia and antipsychotic medication

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Background: Microglia are thought to contribute to the pathophysiology of schizophrenia. Positron emission tomography (PET) has demonstrated elevated microglial signal patients[1], however it is unclear when this first presents. It is also unclear how this microglial activity is affected by antipsychotic medication.

Methods: We use [^{11}C]PBR28 PET to image microglia in patients with schizophrenia, unmedicated high risk for psychosis subjects (HR) and matched controls to determine whether microglial signal is elevated prior to the first episode of psychosis and start of medication.

We also dose rats with haloperidol to study medication independent of psychosis. We test context specificity in naïve and lipopolysaccharide (LPS) rats. We quantify microglial cell changes using confocal microscopy and automated custom software. Microglial changes are compared with peripheral cytokines. To assess mechanisms of changes observed, we investigate apoptosis and proliferation.

Results: HR and schizophrenia subjects demonstrate elevated grey matter (p<0.01), temporal (p<0.01) and frontal (p<0.05) signal, when compared with controls. HR PBR28 signal and symptoms were correlated (r=0.730 p<0.05).

Rats dosed with haloperidol demonstrated a reduction in brain volume (p<0.01) with respect to controls. Microglial density (p<0.01), soma area (p<0.001) and soma stain intensity (p<0.05) were elevated by 50% with LPS. In LPS treated animals, haloperidol restored these to control levels. In naïve animals, these parameters were reduced by 40% with respect to controls. Microglial branches became more complex with LPS treatment, however haloperidol did not restore this. Peripheral CXCL1 and TNFα were elevated by LPS (p<0.01), however in both naïve and LPS animals, haloperidol did not alter levels. There was no difference in apoptosis in microglia, but LPS elevated total number of apoptotic cells (p<0.01), which was reversed by haloperidol. We are currently in the process of assessing proliferation.

Conclusions: Microglial activity, as measured using [^{11}C]PBR28, is elevated in HR and schizophrenia. The evidence from our haloperidol investigation suggests an anti-inflammatory effect of medication, however microglial cell reduction is not attributed to apoptosis. We need to relate the intricacies of our microglial stain evidence to PET signal to determine the true relationship between these measures.

References

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T12-04A

Green tea extract decreases astrogliosis and oxidative stress in the frontal cortex of obese rats

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Obesity induces a chronic and low-grade inflammation in several tissues, including the central nervous system (CNS). Hypothalamic inflammation is an early factor for the onset of obesity, which occurs even before body weight gain. Because of their physical proximity to blood vessels and their function in transporting nutrients, astrocytes are directly affected by hypercaloric diet and produce cytokines that drive inflammatory responses within the CNS. It is known that the polyphenols of green tea (GT) (*Camellia sinensis)* attenuate some deleterious effects of the high-fat diet induced obesity, acting as strong antioxidants and also by reducing CNS inflammation. The purpose of this study was to evaluate the astrocytic response and the oxidative stress in the frontal cortex of obese animals treated with GT extract. For this, male Wistar rats were divided into 4 groups: control (C), green tea (GT), obese (Ob) and obese plus green tea (Ob+GT). The GT and Ob+GT groups received by gavage 500 mg/kg of GT extract for 30 days and then started the high calorie diet (cafeteria diet) for another 60 days accompanied by GT administration. The rats were euthanized and samples of the frontal cortex were collected for immunohistochemical study of astrocytic GFAP expression, evaluation of parameters of the CNS redox status (antioxidant enzyme activity/indicators of oxidative damage) and inflammation (IL-1beta, TNF-alpha, IL-6). Results showed that cafeteria diet increased the astrocytic GFAP immunoreactivity by 56% compared to the control group, whereas obese rats treated with GT showed a 36% decrease in the GFAP expression. Although catalase activity, glutathione peroxidase (GPx) and GSH/GSSG ratio remained unmodified among the groups, MnSOD activity was reduced in obese rats treated with GT compared to obese rats. CuZnSOD was significantly increased after GT treatment by 16% (control vs GT) and 44% (Ob vs. Ob+GT). Glutathione reductase (GR) activity was increased in obese rats treated with GT and glucose-6-phosphate dehydrogenase (G6PDH) decreased in the Ob+GT group by 21% compared with the GT group. Carbonyl groups decreased and free thiol groups increased in the groups supplemented with the extract. In the cortex homogenate the cafeteria diet increased IL-6 release by 108%, whereas obese rats supplemented with GT showed a 40% decrease in this cytokine. No differences were found among groups for IL-1beta and TNF-alpha. Our results showed that GT had some positive effects in reducing the oxidative stress and the astrogliosis in the CNS of obese rats.

T12-05A

IL-4 induces an acute pro-inflammatory burst and activates an alternative gene program mediated by the JAK1/JAK3/STAT6 pathway in microglia

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Objective: The aim of this study was to investigate the signaling pathways underlying activation of an alternative M2 gene program induced by IL-4 in microglia[1].

Material and Methods: Primary cultures of microglia or mixed glia obtained from postnatal mice were treated with murine rIL-4, and dose (1-50 mg/mL) and time (5min-48h) response studies were carried out. JAK inhibitors and STAT6, STAT1, or MYD88 deficient cells were used. Gene expression was investigated by RT-PCR and Affimetrix microarrays. Protein expression was studied by immunofluorescence, Western blotting, or ELISA. Cells were challenged with the protein synthesis inhibitor cycloheximide (CHX, 1mg/mL, 30 min before IL-4).

Results: IL-4 induced the expression of M2 markers, e.g. Arg-1, YM-1, Gal-3, KLF-4, Clec7a, and ITGB3, in a manner dependent on JAK1, JAK3, and STAT6, but not JAK2 or STAT1. CHX prevented the induction of M2 genes suggesting that it was mediated by prior protein synthesis downstream of

STAT6. Using global expression profiling we analyzed whether IL-4 induced changes in gene expression was JAK3-dependent. The analysis identified the JAK3-independent expression of pro-inflammatory genes, such as *TNF-a, IL-1b, IL-1a, Igr-1, and NFkBia*. RT-PCR validation showed that the induction of pro-inflammatory genes was early, mild, and transient, and was downregulated by the JAK1/JAK3/STAT6 pathway. The mRNA expression of pro-inflammatory genes induced by IL-4 was strongly enhanced by CHX, which is known to cause superinduction of early genes by promoting mRNA stabilization[2]. Furthermore, pro-inflammatory gene expression was preceded by a decrease in IkBa protein expression, was dependent on MyD88, and was followed by the release of TNF-a, but not IL-1b, to the medium, suggesting that NFkB, but not the inflammasome, was activated.

Conclusion: IL-4 activates an M2 gene program in microglia mediated by the JAK1/JAK3/STAT6 pathway. However, IL-4 also causes an early and transient induction of pro-inflammatory gene expression that could acutely potentiate the cellular responses to inflammatory or infectious challenges. Inhibition of the JAK1/JAK3/STAT6 pathway not only abrogates the activation of the M2 program, but it stimulates the acute pro-inflammatory burst induced by IL-4.

References:

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T12-06A

Early neuroinflammation biomarkers in the Experimental Allergic Encephalomyelitis (EAE), an animal model for Multiple Sclerosis

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Experimental allergic encephalomyelitis (EAE) is the most commonly used experimental animal model for human multiple sclerosis (MS), which has been used so far to study the acute and remission-relapsing phases of the disease. Despite the neuroinflammation onset studies and its progression in EAE, important questions remain about a possible correlation between tissue neuroinflammation, demyelination, neurodegeneration and potential biomarkers in biological fluids. In this study, we performed a time course investigation of neuroinflammation biomarkers in spinal cord, cerebrospinal fluid (CSF) and blood, using high-throughput technologies for gene expression and protein assays.

Female Dark-Agouti rats were immunized using an emulsion of guinea pig spinal cord, heat-inactivated *Mycobacterium tuberculosis* and complete Freund's adjuvant. Rats were daily weighed and examined for clinical score. At post-immunization days (DPI) 1, 5, 8, 11 and 18, rats were scarified and both biological fluids and tissues were collected. For the spinal cord gene expression, a real-time PCR array of inflammation genes was used. xMAP technology and Luminex platform were used for multiparametric quantification of cytokines and chemokines in CSF and plasma. Several pro-inflammatory cytokines and chemokines such as TNFa, IL1b, CCL5, CCL12, were highly upregulated in the tissue at 8 DPI, reaching a peak at 11 DPI. In particular TNF and IL1b genes were more than 80 fold upregulated at time 11 DPI. CXCL11, CXCL9 and LTA were the most upregulated genes showing 100 fold change at 8 DPI, around 300 at 11 DPI and 125 at 18 DPI. The highest upregulation was observed for IFNg (500 fold change at 11 DPI). Anti-inflammatory cytokines and chemokines were also upregulated at gene and CSF protein level, including VEGF, IL10, GPX1, reaching a peak at 8 DPI then showing a trend to decrease at 11 and 18 DPI. VEGF showed the same gene expression profile as CSF protein level, starting to decrease from 1 to 5 DPI, recovering at 8 DPI and starting again to decrease from 11 DPI. GPX1 gene expression profile was the same as the observed for

VEGF. In plasma, some cytokines and chemokines like IL1b, IL2, CCL5, were upregulated, reaching a peak at 8 DPI while some others started to increase from 1 DPI such as TNFa, IL10 and IL6. This early biomarkers regulation might reflect the immunological response, which starts at peripheral system after 1 DPI, while the blood brain barrier (BBB) is still intact. More delayed regulations in CSF and blood might also include CNS-derived proteins, following to the BBB breakdown.

T12-07A

Microimmunotherapeutic administration of cytokines improve the clinical symptoms in EAE, an animal model of multiple sclerosis

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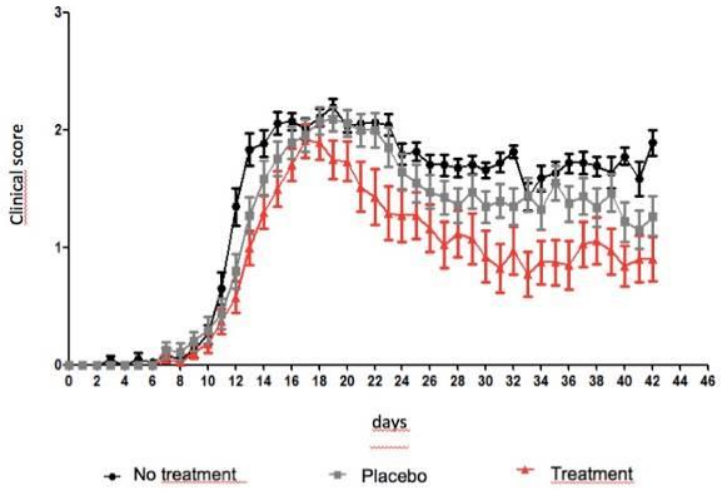
Experimental Autoimmune Encephalomyelitis (EAE) is one of the most used animal models in the study of Multiple Sclerosis (MS). EAE is induced by the injection of myelin proteins and specific adjuvants and leads to an important inflammatory process with activation of resident glial cells, principally microglia, which interact with infiltrated peripheral immune cells, mostly T-cells. In this context, and as described in MS, cytokines, play a crucial role in the cross-talk between these cell populations and in the modulation of the associated neuroinflammatory response. The main objective of our research is to interact in this process by modulating the immune response. Our work hypothesis is that the microimmunotherapeutic administration of specific combinations of cytokines closely related with the neuroinflammatory response may improve the clinical symptoms in EAE. To accomplish that, EAE was induced in C57BL/6 mice by injecting MOG_{35-55} and Complete Freund's Adjuvant supplemented with *Mycobacterium Tuberculosis* and *Pertussis Toxin*. As control some animals were injected with saline. Both, MOG-injected and saline animals, were distributed in three groups: 1) without treatment, 2) treated with placebo and 3) treated with a stimulatory/inhibitory/modulatory combination of cytokines The clinical score of the animals were recorded daily and both the glial response and the infiltration of peripheral immune cells were evaluated using flow citometry and immunohistochemistry.

Our results clearly demonstrated that the group administered with the cytokine combination presented a delay in the onset of clinical symptoms and a significant reduction of the clinical score during the chronic phase of the disease (Fig. 1). These clinical changes correlated with a reduction in the microglial activation pattern and a low number of lymphocytes.

In conclusion, our results suggest that the microimmunotherapeutic administration of specific combinations of cytokines, exert a beneficial effect in EAE progress and could be a very good strategy for modulating the neuroinflammatory response associated with certain CNS-diseases such as MS.

Image



T12-09A

Altered immune signaling (TLR4 deficiency) impairs oligodendrocyte lineage cell responses and functional recovery after spinal cord injury in mice

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Prominent oligodendrocyte (OL) loss occurs after spinal cord injury (SCI), followed by a rebound in the first 2 weeks post-injury due to proliferation and differentiation of surviving OL progenitor cells (OPCs). The mechanisms controlling this endogenous response, however, are not understood. Previous work from our group showed activation of toll-like receptor 4 (TLR4) on microglia induced OPC proliferation and oligodendrogenesis in the intact spinal cord. Since TLR4 ligands are present in the injured spinal cord, we tested the hypothesis that TLR4 signaling contributes to oligodendrogenesis after SCI. Wild-type (WT, HeOuJ) and TLR4-deficient (TLR4d, HeJ) mice were given a midthoracic moderate contusion SCI and sacrificed for spinal cord histology and polymerase chain reaction (PCR) at 1, 3, 7, 14, or 21d post-injury; behavioral analyses and axon and myelin integrity analyses were conducted on a separate set of animals surviving up to 42dpi. Reduced locomotor recovery by the Basso Mouse Scale (BMS) and automated horizontal ladder in TLR4d mice coincided with reduced OL lineage cell responses and less spared tissue chronically in ventral motor tract areas. Compared to WT mice, TLR4d mice had increased OL loss acutely concurrent with decreased ferritin (iron storage) expression in the lesion, suggesting TLR4 signaling helps protect against acute iron-mediated damage. TLR4d mice had reduced OL numbers and OPC markers chronically despite enhanced acute OPC proliferation. Inhibitors of OL differentiation (e.g., Id2, Id4, BMP) were increased in TLR4d mice, likely promoting lower chronic OL numbers. Additionally, delayed lipid accumulation in TLR4d mice, indicative of slower debris phagocytosis, could contribute to reduced OPC and OL responses. We hypothesized that TLR4d mice would have reduced oligogenic growth factor expression. Interestingly, growth factor expression was elevated, including IGF1, CNTF, FGF2 and TGFb, suggesting that TLR4 signaling normally suppresses growth factor expression after SCI. Collectively, this work shows that post-SCI OL protection and replacement are influenced by the inflammatory environment, and highlights the importance of TLR4 signaling for normal post-SCI cellular repair mechanisms, growth factor expression, and functional recovery.

T12-10A

FTY720 attenuates excitotoxicity and neuroinflammation

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FTY720 (fingolimod, Gilenya™), a structural analog of sphingosine-1-phosphate (S1P), is the first oral drug approved for treatment the relapsing-remitting form of the multiple sclerosis, and its efficacy has been related to induced-lymphopenia and immunosuppression via modulation of $S1P_1$ receptors. However, due to its lipophilic nature, FTY720 crosses the blood brain barrier and acts directly on neural cells. Thus, FTY720 has anti-inflammatory effects following CNS injury, reduces pro-inflammatory cytokine production and enhances neurotrophic factor production from activated microglia. However, a direct action of FTY720 on neurons remains unclear. In this study, we used models of excitotoxic neuronal death and neuroinflammation to investigate the effectiveness of FTY720 as a neuroprotective agent, and the potential mechanisms involved. We induced excitotoxicity stimulating primary neuronal and organotypic cortical cultures with NMDA, and found that FTY720 attenuates neuronal demise. In addition, we challenged this drug in a rat *in vivo* model of neuronal death and neuroinflammation caused by intracerebroventricular (*icv*) administration of kainic acid (KA). Daily treatment with FTY720 (administered *icv* together with KA, plus intraperitoneally starting 24h before KA injection) reduced KA-induced damage and microgliosis at the CA3 region. In addition, we tested FTY720 as a modulator of microglia responses using microglial cell cultures activated with lipopolysaccharide (LPS), and its effects in stress signalling pathways p38 and JNK1/2 mitogen-activated protein kinases (MAPKs). We found that FTY720 negatively modulates p38 MAPK in LPS-activated microglia, whereas it has no effect on JNK1/2 activation. We conclude that FTY720 exerts direct protection against excitotoxin-induced neuronal death, and that it is a negative modulator of inflammation by targeting the p38 MAPK stress signalling pathway in microglia which in turn, may indirectly contribute further to limit neuronal loss after injury.

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T12-11A

The abundance of myeloid-derived suppressor cells protects against myelin damage in EAE

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The study of the molecules or cells controlling myelin destruction is a key point in demyelinating diseases like multiple sclerosis (MS). The characteristic symptoms of this disease are consequence of the newly formed lesions within the central nervous system (CNS) and the expansion of older ones. Neuropathology of MS includes lymphocyte and monocyte white matter infiltration, as well as formation of demyelinating areas due to oligodendrocyte loss and axonal degeneration. Currently available treatments for MS rely on immunomodulators that reduce the number of relapses and the subsequent neurological damage, but do not favor the issue of myelin preservation or even remyelination of plaques. The existence of demyelinated plaques where remyelination spontaneously occurs implies the existence of cellular immunomodulatory agents to promote the destruction-to-regeneration transition.

Myeloid-derived suppressor cells (MDSCs) form a heterogeneous population of immature myeloid cells that participate in the suppression of the inflammatory response. In previous studies, our group described the dynamics of MDSCs in the spinal cord of experimental autoimmune encephalomyelitis (EAE) mice. Although absent in control conditions, MDSCs ($Arg\text{-}I^+CD11b^+Gr\text{-}1^+$-cells) transiently enter the spinal cord of EAE to limit the immune response by accelerating T cell apoptosis. To be effective, MDSCs should remain in an undifferentiated state, preventing their polarization to mature myeloid cell subsets.

In the current work, we describe the unexplored relation between the MDSC abundance at both the peripheral immune system and the CNS, with three important histpathological aspects: i) the myelin destruction/preservation degree, ii) leukocyte content within the white matter (neutrophils, lymphocytes) and iii) axonal damage inside and outside the demyelinated area. Finally, we analyze the relationship between MDSCs presence/activity and the aggressiveness of the EAE clinical course.

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T12-12A

SK channels modulate alpha-synuclein-dependent microglial activity and mitochondrial metabolism

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Small conductance calcium-activated potassium (SK) channel modulation is an emerging therapeutic approach for treatment of neurological diseases, including stroke, amyotrophic lateral sclerosis and neurodegenerative diseases, such as Parkinson's disease. Our previous studies showed that activation of SK channels in neurons exerted protective effects through inhibition of NMDAR-mediated excitotoxicity. Further, we revealed recently that SK channels are also located at the inner mitochondrial membrane of neuronal mitochondria. In a model of glutamate toxicity, activation of SK channels attenuated mitochondrial fission, prevented the release of pro-apoptotic mitochondrial proteins, and reduced cell death. However, little is known about the function of SK channels in cell metabolism and neuroinflammatory processes in non-neuronal cells, such as microglial cells. In this study, we addressed the question whether SK channel activation affected inflammatory responses of primary mouse microglia upon α-synuclein challenge. We found that activation of SK channels significantly reduced activation of microglia in a concentration-dependent manner, as detected by real-time xCELLigence cell impedance measurements. Interestingly, α-synuclein-induced glycolysis was reduced by SK channel activation as detected by the cell metabolism Extracellular Flux Seahorse analyzer and metabolite measurements. Further data on multi-array cytokine analysis revealed that activation of SK channels attenuated α-synuclein-induced cytokine release. Inhibition of glycolysis prevented microglial activation and cytokine release. Although SK channel activation slightly reduced ATP levels, it attenuated α-synuclein-induced NO release. Electrophysiology recordings demonstrated that membrane SK channels play a minimal role in alpha-synuclein-mediated microglial activation, suggesting that SK channels located at intracellular sites might be responsible for the attenuation of microglial activation. Thus, SK channels are promising therapeutic targets for neurodegenerative disorders such as Parkinson's disease, where neuroinflammation and cell metabolic deregulation are associated with progression of the disease.

T12-13A

Differentially activated microglia release Extracellular Vesicles (EVs) presenting specific contents and functions in a model of nerve repair

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The leech *Hirudo medicinalis* is a well-studied model in neurobiology because it undergoes synapse regeneration as a natural and functional process. Within 24 hours following a crush in the connectives, resident microglial cells migrate at the lesion. When microglial accumulation is inhibited, a significant reduction in axonal sprouting on damaged neurons occurs, showing that microglia are essential for the natural repair of injured axons. Resident microglia are the "immune triggers" to the synapse regeneration since we know that no blood cell infiltrate the CNS. Of interest, regeneration is mostly mediated by microglial cells because there are neither astrocyte nor oligodendrocyte in leech CNS.

While the importance of infiltrating macrophages is highly questioned in neurodegenerative diseases, the understanding of resident microglia is a necessary prerequisite to elucidate successive pathogenic steps. Our results showed that the microglial mobility associates specific factors including leech homologs for C1q, EMAPII and/or Interleukin-16 allowing microglial recruitment to the lesion. The characterization of specific markers in the leech suggested that microglial subpopulations may be differentially recruited according to chronological and functional features. Thus our data highlight the involvement of several activating and migrating signals acting on different microglia subsets at the lesion.

A massive production of extracellular vesicles (EVs) has been observed from leech microglia accumulated at the lesion site allowing a crosstalk with damaged neurons. In order to define the functional properties of microglial cells recruited to lesioned leech CNS, EVs produced by microglia were collected, purified and their molecular contents were analyzed using proteomic and transcriptomic approaches. The results show that EVs contain differential molecular pattern according to the microglial activation through ATP, C1q, EMAPII or IL-16. In addition these differentially produced EVs were also tested for their capacity to interfere with the outgrowth of damaged neurons suggesting that EVs trigger and mediate the neuroprotective action exerted by microglial cells on neurons.

Thus the characterization of a neuroprotective microglial phenotype in the leech CNS would help to understand processes promoting nerve repair. As in mammals, neuroprotective microglia may be followed by the identification of novel molecular markers and the study of their functions will be necessarily associated with the analyses of specific EV signatures.

T12-14A

Profiling glial CXCL12 receptor expression during experimental autoimmune encephalomyelitis

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The chemokine CXCL12/SDF-1 induces and modulates major steps of ontogenesis, regeneration, tumorigenesis, and inflammation. In the CNS, CXCL12 and its alleged primary receptor, CXCR4, are indispensable for proper brain development, and are upregulated following injury to promote regenerative processes. However, information about the function of the recently identified second CXCL12 receptor, CXCR7 (AKCR3), in neural tissue is still sparse. To elucidate the roles of CXCR7 and CXCR4 in glial cells, we have initially analyzed the effects of CXCL12 on cultured primary rodent astrocytes and microglia. It turned out that CXCL12-induced ERK phosphorylation and proliferation of astrocytes is solely evoked by the CXCL12-CXCR7 axis, whereas microglia need both receptors for signal transduction. Since proliferation/activation of these glial cell types is a typical feature of central nervous (auto)immune disorders such as multiple sclerosis, we have now investigated the protein expression patterns of CXCR4 and CXCR7 in the spinal cord of rats with experimental autoimmune encephalomyelitis (EAE). In wild-type animals CXCR4-immunostaining was extremely faint in microglia and astrocytes. Induction of EAE remained without obvious effects on CXCR4 expression in both glial cell types, however, resulted in the appearance of an additional population of CXCR4-immunoreactive cells, which showed double-labelling for the microglial/monocyte marker, Iba-1, and exhibited a round monocyte-like morphology. In control spinal cords, CXCR7 was only sparsely expressed by astrocytes and virtually absent from microglia. Unlike CXCR4, CXCR7 was clearly upregulated in both cell types after induction of EAE, but remained undetectable in monocyte-like cells. Our findings imply that CXCL12 primarily controls glial function via CXCR7 under pathological conditions. In addition, our findings point to CXCR4 as the major CXCL12 receptor present in monocytes entering the injured brain.

T12-15A

Activation of the alternative (RelB-dependent) NF-kB pathway in microglia is required for brain inflammation in experimental autoimmune encephalomyelitis

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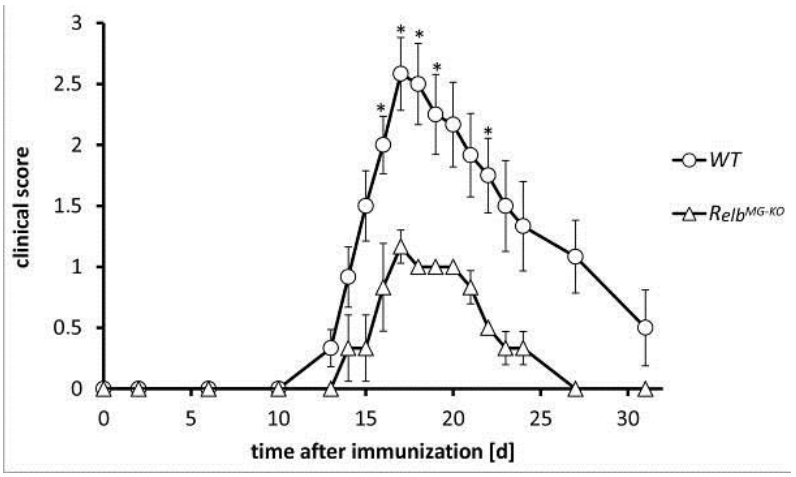
Microglia derive from the myeloid cell lineage and represent the innate immune system of the brain. They are required for tissue homeostasis and react to acute injuries and chronic diseases of the CNS. Resting microglia are activated by cytokines released from injured cells or activated T_H17 cells, resulting in a transformation to scavenger and immune effector cells. In contrast to the well-established function of classical NF-κB signaling (activating RelA/p50 transcription factor complexes) for microglia activation, the role of the alternative NF-κB pathway (activating RelB/p52 complexes) for brain inflammation remains undefined. Previously, we showed expression of RelB and its dimerization partner p52 in primary cultures as well as freshly isolated microglia from mice. Here, we studied the activation of RelB in response to microglial stimulation and elucidated the functional consequences of microglia-specific depletion of RelB in the context of brain inflammation *in vivo*.

Using confocal microscopy and immunocytochemistry to analyze the intracellular localization of RelB in microglial cultures we found that in naïve cells RelB was primarily located in the cytoplasm. Stimulation for eight hours with an agonistic lymphotoxin β receptor antibody resulted in nuclear translocation of RelB indicating an activation of the alternative NF-κB pathway in microglia.

To elucidate the activation of alternative NF-κB signaling and the microglial functions of RelB under pathological conditions we crossed inducible *CX3CR1-CreER^{T2}* mice (Yona *et al.*, Immunity 38, 2013) with *Relb^{flox}* mice and induced experimental autoimmune encephalomyelitis (EAE) by injection of myelin oligodendrocyte glycoprotein (MOG) peptide. Microglia-specific deletion of *Relb* (*Relb$^{MG\text{-}KO}$*) in the presence of *Relb$^{+/+}$* peripheral monocytes was ensured by a four-week recovery period of the peripheral immune compartment after tamoxifen treatment. Quantitative assessment of clinical scores in EAE-induced mice revealed a significant reduction of neuropathological symptoms in *Relb$^{MG\text{-}KO}$* mice (peak score: 1.2 ± 0.1) compared to control mice (peak score: 2.6 ± 0.3; $P < 0.05$; Fig. 1). To reveal changes in the inflammatory response we are currently characterizing the antigen-specific autoreactive T cell infiltration and the microglial activation in the brains of *RelB$^{MG\text{-}KO}$* mice during EAE progression by *in vitro* re-stimulation experiments followed by FACS analysis. In summary, our results show an activation of the alternative NF-κB pathway in microglia and its pivotal involvement in CNS inflammation.

Figure 1. Microglia-specific deletion of RelB ameliorates the severity of neuroinflammation in experimental autoimmune encephalomyelitis (EAE). Clinical scores of *Relb$^{MG\text{-}KO}$* and *wild-type* (*WT*) mice. $P < 0.05$.

Image



T12-16A

Sulforaphane exerts protective effects in microglial cells by switching polarization phenotypes

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Introduction: Microglial cells are the brain macrophages and constitute approximately %10-20 percent of glial cell population in the brain. They become activated in response to injury or pathological conditions. Neurodegenerative diseases such as Alzheimer's disease and Parkinson's disease are linked to activated microglia. Similar to peripheral macrophages, microglial cells also exhibit different cell polarization phenotypes. The so-called M1, M2, and Mox phenotypes are distinguished by their activation mechanisms and unique gene signatures. The Mox phenotype is particularly interesting due to its association with distinct gene expression profile.

Question: We hypothesized that sulforaphane may inhibit microglial polarization status M1 and may induce Mox phenotype.

Method: Real-time RT-PCR was performed to analyze microglial activation status markers.

Results: Here, we show that sulforaphane (SFN), a phytochemical found in cruciferous vegetables, protects microglial cells from LPS-induced inflammation by switching cell polarization phenotypes. We found that SFN pretreatment decreased mRNA expression levels of M1 markers interleukin 1 beta (Il-1b), Il-6, inducible nitric oxide synthase (iNOS), and tumor necrosis factor alpha (TNF-a). At the same time, we found increased mRNA expression levels of Mox markers, heme oxygenase 1 (Hmox1) and sulfiredoxin 1 (Srxn1). On the other hand, SFN had no significant effect on M2 markers.

Conclusion:Taken together, our results suggest that SFN exerts its protective effects by suppressing M1 phenotype, and switching to the Mox phenotype.

T12-17A

The role of microglial P2Y12 in controlling neurotrophic virus infection in the brain

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Understanding immune mechanisms that are initiated against viral infections in the CNS is essential to develop appropriate therapies to diseases such as HSV enchephalitis or viral meningitis. Microglial cells are known to be rapidly recruited to the sites of brain injury and we have demonstrated earlier that microglia form barriers around virus infected cells in the brain. However, the functional role of microglia in defense against neurotrophic viral infection and mechanisms controlling microglia recruitment to infected neurons are not understood. We have established in vitro cultures of microglia and pseudorabies virus (PRV)- infected neurons / astrocytes, and found that microglial recruitment to infected cells is associated with increased Ecto-ATPase activity, which was also confirmed in the infected mouse brain, in vivo. To study whether ATP could mediate the recruitment of microglial cells to sites of neurotrophic viral infection in vivo, we injected a highly attenuated, Bartha-derived PRV strain into the epididymal fat of wild type, P2X7 and P2Y12 knock out (KO) mice. In this experimental model viral particles are retrogradely transported to central autonomic neurons in the brain and the genetically modified pseudorabies virus strain used allows the assessment of early and late stages of neuronal infection. Microglia were rapidly recruited to infected neurons in the brain and phagocytosed cells showing signs of late infection. We found reduced microglia recruitment around infected neurons in P2Y12 KO mice, which was associated with enhanced spread of viral infection in P2Y12 KO, but not in P2X7 KO mice. Our results suggest that ATP released from infected neurons and/or nearby cells

could mediate rapid recruitment of microglia to the sides of neurotrophic viral infecion in the brain. Therapeutic interventions facilitating the phagocytosis of virally infected neurons by microglia could be used to support the elimination of neurotrophic viruses and infected neurons from the CNS.

T12-18A

High fat diet effects on brain inflammatory mechanisms and hypothalamic progenitor cells

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Global obesity rates are on the rise and though there are multiple key factors, such as diet and lifestyle that interact, ultimately an alteration of the feeding circuitry seems to underlie chronic deregulation of feeding behavior. A recent study showed that consumption of diets high in fat could induce brain inflammation. A hallmark of neuroinflammation regardless of the trigger is the activation of microglia - the resident immune cells in the brain. Microglia are known to phagocyte viable neurons during a neuroinflammatory event and coincidently, after high fat diet, there is also an increase in cell death of newly born neurons in the hypothalamus, leading to impaired remodeling of this structure and possibly perpetuating deregulation of feeding behavior. We hypothesize *that microglia are involved in the phagocytosis of these newly born neurons* and the aim of the present study was to determine if consumption of high fat diet alters the microglial phenotype and function. In particular we explored the nature and level of interaction between microglia and neural progenitor cells in the hypothalamus and whether these are also affected early in diet-induced obesity before weight gain is evident.

T12-19A

DNGR-1+ dendritic cells are located in meningeal and choroid plexus membranes of the non-injured brain

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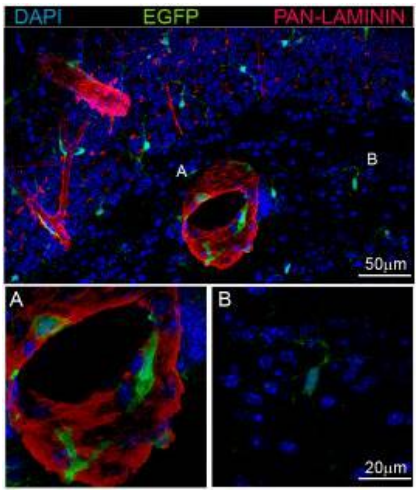
The role and different origin of brain myeloid cells in the brain is central to understanding how the central nervous system (CNS) responds to injury. C-type lectin receptor family 9, member A (DNGR-1/CLEC9A) is a marker of specific DC subsets that share functional similarities, such as CD8a+DCs in lymphoid tissues and CD103+CD11blowDCs in peripheral tissues. Here, we analyzed the presence of DNGR-1 in DCs present in the mouse brain (bDCs). Dngr1 mRNA is expressed mainly in the meningeal and choroid plexus (m/Ch) membranes, and its expression is enhanced by fms-like tyrosine kinase 3 ligand (Flt3L), a cytokine involved in DC homeostasis. Using Clec9agfp/gfp transgenic mice, we show that Flt3L induces accumulation of DNGR1-EGFP+ cells in the brain membranes. Most of these cells also express major histocompatibility.

Our results support a dendritic-like nature for Flt3L-dependent cells in the brain. First, they are responsive to Flt3L, a cytokine that has been shown to increase the dendritic repertoire. Second, they express DNGR-1, indicated by examination of Cle9agfp/gfp mice and the expression of Dngr1 mRNA. Finally, they express the transcription factors Batf3 and Irf8, both of which are required for the maturation of CD8α+ classical DCs (Murphy et al., 2013).

The existence, regulation and role of APCs in the brain DCs, has implications for the development of strategies to target cargoes to these m/Ch brain DCs using receptor-specific antibodies. The expression of the highly specific receptor DNGR-1 in these cells makes such strategies especially attractive The similarity of these cells to lymphoid CD8a+DNGR-1+ cells suggests that they might be responsible for producing efficient CTL responses (Caminschi et al., 2008; Sancho et al., 2008), and

therefore could be an important target in brain disorders such as neuroinflammation-based neurodegenerative diseases, viral-induced encephalitis and brain tumours such as gliomas.

Image



T12-20A

Chronic cortical inflammation as a novel experimental model of progressive Multiple Sclerosis. Influence of the innate immune system

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Multiple Sclerosis (MS) is a neurodegenerative disease characterized by repeated inflammatory events, demyelination and axonal damage, along with loss of function. MS exhibited different forms: relapsing remitting (RRMS), primary and secondary progressive (PPMS, SPMS). Even though neuroinflammation is hallmark in every form of the disease, immunomoduladory treatments are beneficial in the early stages of MS, but ineffective in PPMS and SPMS. Recently cortical lesions were described in PPMS and SPMS patients, which contribute to physical disability and cognitive impairment that characterize the progressive forms. The pathogenesis of cortical lesions is still unknown. Therefore, the cortical microenvironment, could influence the degree of inflammation, tissue damage and the repair of the lesions.

We developed a model of chronic and focal inflammatory triggered by the long term expression of one inflammatory cytokine, interleukin-1beta (IL-1b). Additionally, regional differences to the long term expression of IL-1b, were found between striatum and *Substantia Nigra*.

The aim of this work is to study the effect of the chronic expression of IL-1 in the cortex of adult rats and the effect of peripheral pro-inflammatory stimulus on these lesions. We used an adenovector expressing human IL-1b (AdIL-1b) or betagalactosidase (Adbgal) to induce chronic expression in the cortex. We performed behavioral, histological, immunohistochemical and molecular analysis.

The long term expression of IL-1 in the cortex induces inflammation characterized by neutrophil recruitment and edema, neurodegeneration, and astro and microglia activation. The inflammation peaked at 15-21 days post injection and the lesion is restored by 30 days after injection. These results are correlated with a worse performance in the behavioral test of novel object recognition of IL-1 injected animals at the peak of inflammation (15-21 days), which is improved as far as the lesion is recovered (30 days). The peripheral stimulation exacerbates the cortical lesion, with an increased inflammatory and glial response.

In the present work, we developed an experimental model of cortical inflammation and cognitive deterioration. The details of the pathophysiology of the progressive MS need to be better understood. A simple animal model allows the analysis of individual components of MS pathology, which could be use as targets for designing specific progressive MS treatments.

T12-21A

Effect of an omega-3/antioxidants supplemented diet on emotional and cognitive alterations and neuroinflammatory processes associated with obesity

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Obesity is a metabolic and inflammatory disorder that represents a major risk factor for development of comorbidities such as cardiovascular diseases or diabetes. It is also associated with an increased prevalence of mood disorders and cognitive dysfunctions that represent important risk factors for aggravation of obesity and related outcomes. Reducing the development of such disorders in the context of obesity may be therefore a way to improve health and quality of life of obese subjects. Converging clinical and experimental studies have suggested that inflammatory processes, which are often associated with severe obesity, might contribute to development of mood and cognitive alterations, in particular when they occur in brain areas associated with mood, learning and memory such as the hippocampus. Interestingly, nutriments such as n-3 polyunsaturated fatty acids (n-3 PUFAs) or antioxidants (AO) have been shown to protect against the development of emotional and cognitive alterations in different inflammatory conditions and are potent modulators of neuroinflammation. However, their effect in the context of obesity still remains relatively unknown. Thus, the aim of this study was to evaluate: 1) if a diet supplemented with n-3 PUFAs and AO was able to improve anxiety-like behavior and cognitive deficits displayed by a genetic mouse model of obesity (*db/db* mice), and 2) if this potential behavioral improvement was associated with reduced inflammation. Chronic consumption (12 weeks) of the supplemented diet did not improve anxiety-like behavior (light/dark box, open-field) but it reversed hippocampus-dependent spatial memory deficits displayed by control *db/db* mice in a water maze task. Studies are now in progress to assess the impact of the supplemented diet on peripheral and brain inflammation by respectively measuring circulating cytokines levels and hippocampal expression of inflammatory and anti-inflammatory cytokines, as well as activation of their intracellular signaling pathways. These findings should provide valuable data for introducing new nutritional strategies for the treatment of behavioral complications associated with obesity by targeting inflammatory processes.

T12-22A

Characterization of inflammatory response after mouse spinal cord injury

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Inflammatory response plays an essential role to protect the body after injury. Inflammation, however, must be a highly regulated response, otherwise, it may lead to tissue damage and/or to chronic inflammation, as observed after spinal cord injury (SCI). Despite the contribution of inflammation to SCI, the recruitment of the different immune cell populations and the expression at the protein levels of cytokines and chemokines in the mouse contused spinal cord has not been fully characterized. Understanding the factors that impede the clearance of immune cells after SCI is likely to be critical for the development of new therapeutic strategies. Herein we assessed the changes in the main inflammatory cell types and cytokine/chemokine expression in the mouse contused spinal cord.

Our results show that neutrophils are the earliest inflammatory cells to invade the injured spinal cord. They peak at 24 hours post-injury and decline progressively up to day 28. Microglial cells peak at day 7 post-injury and slowly decline up to day 28. The infiltration of monocytes peaks in the contused spinal cord at day 3, and decreases in numbers up to day 14. Few lymphocytes are present within the

injured spinal cord for the first week. Strikingly, there is a great influx of lymphocytes into the contused spinal cord at day 21, and their proportion remain elevated up to day 28. The increase of lymphocytes at later stages of spinal cord injury is mainly due to the recruitment of B cells, and to minor degree, to the invasion of CD4 and CD8 T cells.

Regarding cytokine/chemokine expression, our results reveal that all these pro-inflammatory mediators are up-regulated within the first 24 hours, peaking between 6 and 12 hours post-injury. IL6 and G-CSF are the most abundant cytokines at this early phase, and could play a key role in triggering the activation of glial cells and the recruitment of granulocytes. Beyond 24 hours, most of the cytokines were undetectable, except for some of them, such as M-CSF, IL-1α and IL9, that remain elevated until 28 days. Interestingly, the expression of M-CSF peaks at day 3 post-injury, suggesting its importance in monocyte and microglia expansion in the contused spinal cord. Contrary to most cytokines, the expression of chemokines remains at high levels for the first 3-7 days, and some of them, such as CXCL9, CXCL10 and eotaxin, are overexpressed up to day 28.

In conclusion, our data suggest that the lasting up-regulation of some cytokines and chemokines in the injured spinal cord might be responsible, in part, in the failure of immune cell clearance.

T12-23A

CX3CR1 delection restricts inflammatory signaling in microglia and promotes axon sprouting and synapse preservation after spinal cord injury

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Spinal cord injury (SCI) is characterized by disruption of descending and ascending axonal pathways leading to loss of motor, sensory and autonomic function. Signaling of microglia/macrophages via chemokines, cytokines and other molecules in the lesion microenvironment activates inflammatory functions that are detrimental to axonal growth/ regeneration. Previously, our lab showed that impaired CX3CR1 signaling in microglia/macrophages was associated with reduced neurotoxicity and improved recovery of locomoter function in a mouse model of spinal contusion injury (Donnelly et al., 2011). Although we attributed the improved recovery in these mice to enhanced sparing of axons at the site of injury, we also predicted that without endogenous CX3CR1 signaling, microglia/macrophages create a microenvironment that favors the growth/ preservation and/or plasticity of locomotor circuitry. To test this hypothesis, sixty male or female $CX3CR1^{+/+}$ or $CX3CR1^{-/-}$ mice were anesthetized then subjected to a moderate SCI (75 kdyn IH device). After 4, 14, 28 or 56 days, mice were euthanized and the spinal cords processed for immunohistochemistry, Golgi-Cox staining and transmission electon microscopy (TEM). *In vitro* assays of cortical neonatal neurons and microglia from $CX3CR1^{+/+}$ and $CX3CR1^{-/-}$ mice showed that CX3CR1 deficiency significantly impairs inflammatory signaling in microglia which promotes neuron survival and/or neuroprotection. *In vivo* studies showed that $CX3CR1^{-/-}$ microglia/macrophages co-localize with increased numbers of NG2+ glia at the lesion site. Importantly, the enhanced expression of NG2 proteoglycan in the lesion of $CX3CR1^{-/-}$ mice was especially permissive to growth/sprouting of serotonergic 5HT+ axons. In $CX3CR1^{-/-}$ mice, microglia also exhibt a reduced inflammatory phenotype several segments distal to the lesion. In the lumbar spinal cord, $CX3CR1^{-/-}$ microglia co-localized with significant preservation of serotonergic axons and increased synaptic contacts on ventral horn motor neurons (MNs); ~53% of $CX3CR1^{-/-}$ MNs were covered with synpases while ~41% of MNs contained synapses in $CX3CR1^{+/-}$ mice. Together, these data indicate that blocking CX3CR1 signaling modulates the normal course of inflammation, creating a favorable microenviroment at and below the lesion, in which tissue injury is minimized and axon growth or sprouting can be enhanced. Therefore genetic or pharmacologic manipulation of intraspinal microglia/macrophages by CX3CR1 could be useful for promoting recovery after traumatic SCI.

T12-24A

CD14 control over microglial TLR4 functions involves an IFNß-mediated feedback mechanism

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Microglia are the innate immune cells of the central nervous system (CNS). They are capable of sensing infection and damage through various receptors and consequently trigger an appropriate immune response. In this regard, Toll-like receptor 4 (TLR4) can signal upon recognition of both pathogen-associated molecular patterns (PAMPs), like bacterial lipopolysaccharide (LPS), and damage-associated molecular patterns (DAMPs), such as fibronectin. We had previously shown that microglial TLR4 signaling in response to PAMPs and DAMPs is distinctly organized and that the TLR4 co-receptor CD14 has important regulatory functions. CD14 is mandatory for TLR4-mediated microglial responses to CNS damage, as demonstrated by the lack of microglial reactions to fibronectin in the absence of CD14. Additionally, CD14 keeps microglial responses to infectious challenges in a moderate range. It increases cell-specifically the sensitivity of microglia to low amounts of LPS and protects against overshooting responses to high amounts of LPS. CD14 especially suppresses overproduction of the chemokine CXCL1, thereby preventing excessive neutrophil infiltration into the brain. The regulation of the CXCL1 production depends on an interferon β (IFNβ)-mediated feedback mechanism, which involves the interferon-α/β receptor (IFNAR) and downstream components of the JAK-STAT signaling pathway, including different kinases and transcription factors. In the absence of CD14, the production of IFNβ is massively impaired leading to loss of the negative regulation of the CXCL1 production. Microglia deficient in IFNAR, available by an *ifnar1*$^{-/-}$ strain, show an overproduction of CXCL1 upon stimulation with a variety of PAMP- and DAMP-related TLR agonists, arguing for an important regulatory function of IFNAR signaling in general.

T12-25A

A phosphorous-based dendrimer with anti-inflammatory properties towards microglia

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Microglia, the resident immune cells of the central nervous system, are involved in brain homeostasis but also in neurodegenerative processes, during a hyperactivation state called "reactive microgliosis." The consequences of this microgliosis are, on the one hand, the sustained release of inflammatory mediators with devastating effects on the brain parenchyma and on the other hand, the reduction in the production of neurotrophic and neuroprotective factors. Thus, this reactive microgliosis could act as "catalysts" of brain damages and contribute to the pathophysiology of NeuroDegenerative Diseases (NDD). Indeed, reactive microgliosis and inflammation-mediated neurotoxicity are closely related and are typical hallmarks of NDD, like Alzheimer's and Parkinson's diseases. Despite a century of considerable research, no cure currently exists for these pathologies. Our current project aims at meeting the medical need for innovating therapeutics to treat them. An effective treatment for NDD could be based on the control of this deleterious phenotype. Currently, we are developing an innovative nano-molecule which could fulfill this function.

Dendrimers are a class of highly branched “tree-like” polymers, characterized by their precisely defined structure and molecular weight. Dendrimer multivalency enables polyvalent interactions with cellular and molecular targets; therefore, they have been explored for their potential use in drug delivery, biological imaging, and nanomedicine. In addition, we have shown that an azabisphosphonate (ABP)-capped dendrimer selectively targets monocytes and directs them toward anti-inflammatory activation. We have also provided preclinical Proofs of Concept of the therapeutically efficacy of this dendrimer to fight against inflammation in mouse models of Chronic Inflammatory Diseases (Rheumatoid Arthritis and Multiple Sclerosis). Therefore, we propose that ABP dendrimer could re-educate reactive microgliosis, and thus become a drug candidate for the establishment of a neuroprotective state.

More recently, we have explored the biological properties of this dendrimer on the microglial cell line, BV2. The results show that ABP binds to and drives BV2 cells towards an anti-inflammatory state. More interestingly, ABP is able to reverse an LPS-induced classical M1 inflammatory phenotype. These results strengthen the potential of ABP dendrimer as an innovative nano-sized drug-candidate for the treatment of chronic inflammatory diseases of the central nervous system.

T12-26A

Microglia tolerance to LPS is mediated by RelB-dependent epigenetic silencing

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Questions: Recent evidence indicated that innate immune cells can display immunological memory. Exposure of innate immune cells to a microbial agent can lead to a significantly attenuated or enhanced inflammatory response to subsequent exposure, termed endotoxin tolerance (ET) or trained immunity, respectively. Microglia are the principle innate immune cells of the central nervous system (CNS) and monitor their surroundings for homeostatic disturbances. The aim of this study was to investigate if microglia also display ET and whether that is accompanied by functional and epigenetic alterations.

Methods: Endotoxin tolerance was induced by lipopolysaccharide (LPS) in primary microglia and *in vivo* in mice and determined by reduced inflammatory cytokine expression and secretion (quantitative RT-PCR and ELISA). Chromatin immunoprecipitation (ChIP) was used to check histone modification enrichment and RelB binding in gene promoters. Microglia functions were evaluated by using assays for phagocytosis, electrophysiology, cell morphology and reactive oxygen species(ROS).

Results: We observed that a single challenge with LPS resulted in a prolonged blunted pro-inflammatory response of microglia to a subsequent LPS stimulation, both in primary cultures and *in vivo*. ChIP experiments showed that LPS preconditioning of microglia resulted in an increased enrichment of a repressive histone modification (H3K9me2) and a reduced enrichment for active histone marks (AcH3 and H3K4me3) on the IL-1β promoter. ChIP and loss-of-function experiments showed that NF-κB subunit RelB was bound to the IL-1β promoter in preconditioned microglia and that RelB was required for the observed attenuated LPS response. Preconditioned microglia furthermore displayed enhanced basal phagocytic activity, improved ROS production and increased outward potassium currents in response to LPS.

Conclusion: Our data show that LPS preconditioned microglia display an ET-like phenotype, which is epigenetically regulated and RelB-mediated.

T12-27A

Autoantibody-driven astrocytopathy: creation of an *in vivo* model to decipher the pathophysiological mechanisms of Neuromyelitis optica

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Devic's Neuromyelitis Optica (NMO) is now considered an autoimmune primary astrocyte disease related to central nervous system inflammation, demyelination and neuronal injury. NMO is associated with a specific serum autoantibody targeting the water channel aquaporin 4 (AQP4) mainly expressed on astrocyte. The pathogenicity of AQP4-Ab/NMO-IgG through complement-dependent toxicity to astrocyte was demonstrated. However, autoantibodies can also act as receptor agonists/antagonists or modulate antigen density on their target cells, thus trigger cell dysfunction, prior or in addition to complement activation or secondary inflammatory cell-mediated response. We assume that these mechanisms are involved in NMO pathogenesis.

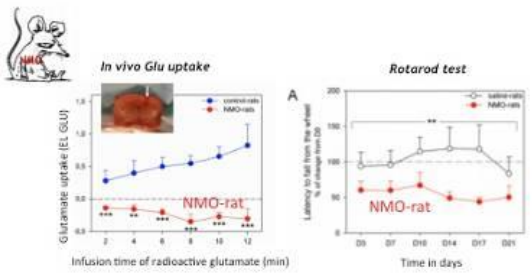
To explore the intrinsic effect of NMO-IgG, we have developed a novel and unique animal model of NMO based on a chronic infusion (7 days, 1µL/h; 300µg IgG) of purified IgG from patient (NMO-rat) and healthy individual (Control-rat), into the brain ventricle of rats. We preliminary controlled that

AQP4-Ab/NMO-IgG binds to AQP4 in rat astrocyte. This approach using “physiological” IgG amount allows investigating autoantibody-mediated pathology in the rat CNS, independently of the activation of additional effectors mechanisms. Infused NMO-IgG was detected in the rat brain, spinal cord and optic nerves and in the blood, confirming the validity of the model.

We found that chronic CNS infusion of purified NMO-IgG, but not control-IgG, induced NMO-like lesions, associating 1- loss of AQP4 with preserved astrocytes mainly in spinal cord and the optic nerve; 2- myelin alteration and axonal loss; 3- motor skills impairment; 4- alteration of glutamate homeostasis as observed *in vivo,* on vigil rat, with a dramatic reduction of glutamate uptake, reflecting the dysfunction of glutamate transporters GLAST and GLT1.

In conclusion, this model confirms *in vivo* the potential modulatory effect of AQP4-Ab/NMO-IgG in the absence of complement activation, as we previously observed *in vitro* (Marignier et al, 2010). Autoantibody-driven astrocytopathy in perfused rat results in NMO-like CNS lesions, glutamate homeostasis disturbance and motor deficits. More generally, our work brings to light some pathomechanisms involved in autoimmune CNS disorders.

Image



T12-28A

Modulation of the glial niche by the neuropeptide Cortistatin: involvement in neuroinflammation and neurodegeneration

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Background: Glial cells play a critical role in brain homeostasis by producing trophic factors, by regulating glutamate-excitotoxicity and by removing cell debris. Thus, acute inflammatory mechanisms mediated by glial cells limit injury and promote neuronal survival. However, dysregulation of glial activities induces an unbalance between trophic/survival support and inflammatory activation. This leads to a neuroinflammation-derived oxidative stress, cytokine-dependent toxicity and damage of neural targets characterizing the processes accompanying the development of progressive neurodegenerative diseases.

Question: Identifying agents able to deactivate glial responses and to trigger directly and/or indirectly neuroprotective mechanisms are highly desirable to design treatments for neurological disorders. Cortistatin (CST), a cyclic-neuropeptide produced by brain cortex and immune cells, shows immunomodulatory and neuroprotective effects in cell-based systems and pre-clinical models of ischaemia, glutamate-induced excitotoxicity and bacterial meningoencephalitis. We have recently reported that CST provides a effective therapy for a pre-clinical model of multiple sclerosis, in which it impaired the autoimmune and inflammatory responses. However, its role in the dynamics and activation of glial cells during neuroinflammatory processes is unknown.

Results: Here, we show that CST deactivated the inflammatory response of resident glial cells, but, at the same time, it induced their CNS supporting roles keeping intact the phagocytosis and trophic functions of these cells. In addition, using glial cells isolated from CST-deficient mice, we demonstrated that lack of CST affected the growth/differentiation of oligodendrocytes and induced an activated phenotype in microglia and astrocytes, which showed exacerbated and dysregulated responses to inflammatory stimulation. Of note, CST levels are significantly decreased in the CNS of animals with experimental autoimmune encephalomyelitis, in the temporal lobe of Alzheimer´s patients, and in the retina of diabetic patients (in which low amounts of CST correlated to increased retinal neurodegeneration and glial activation).

Conclusions: Together, these results support the potential of CST in modulating neurodegeneration in an inflammatory milieu, combining the downregulation of neuroinflammation with trophic support and neuroprotection. Moreover, our findings suggest a crucial role for endogenous CST as a normalizing factor for the glial niche.

T12-29A

Culturing adult mouse microglia

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Microglia, the resident macrophages in the central nervous system (CNS) play a variety of roles during development, normal tissue homeostasis, defence against pathogen and response to injury and disease. Dissociated cultures of CNS cells has led to a greater understanding of all CNS cell types. A major caveat of such cultures is that cells are often isolated from perinatal animals. However, recent transcriptome data shows neonatal microglia have a significantly different profile to those isolated from the adult mouse, and that transforming growth factor beta (TGF-β) and Macrophage colony-stimulating factor (M-CSF) are needed for cultured adult microglia to retain their *in vivo* profiles. Here, we describe a simple protocol to extract and culture adult microglia with high purity using antibody-coated magnetic beads. We cultured isolated microglia from 12 week old mice in DMEM-F12 media containing FBS (10%) with combinations of recombinant M-CSF, conditioned media from L-929 cells (rich in M-CSF) or TGF- β, for 7 days. We find that L-929 conditioned media plus TGF- β provides the highest yield of microglia after 7 days in culture. Microglia under these conditions show a highly ramified morphology compared to those cultured in L929 media or M-CSF alone, and retain a similar transcriptional profile to freshly isolated microglia. These studies characterise an accessible protocol for the culturing adult microglia that allow the study of these cells in a variety of scenarios, including diseases of the adult CNS.

T12-30A

***Ccr2* deletion dissociates cavity size and Tau pathology after mild traumatic brain injury**

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Millions of people experience traumatic brain injury (TBI) as a result of events including falls, car accidents, sports injury and blast. TBI has been associated with the development of neurodegenerative conditions such as Alzheimer's disease and Chronic Traumatic Encephalopathy. In the initial hours and days, the pathology of TBI comprises neuronal injury, breakdown of the blood-brain barrier, and inflammation. At the cellular level, the inflammatory reaction consists of reaction of brain-resident microglia and infiltration of peripheral cells. After TBI, signaling by chemokine (C-C motif) ligand 2 (CCL2) to the chemokine (C-C motif) receptor 2 (CCR2) is a key regulator of brain infiltration by monocytes. In this study, we utilized mice with one or both copies of *Ccr2* disrupted by red fluorescent protein (*Ccr2*$^{RFP/+}$ and *Ccr2*$^{RFP/RFP}$) to examine the effects of altered monocyte entry into the brain on several pathological outcomes in the fluid percussion model of TBI. *Ccr2* deletion reduced monocyte infiltration, diminished lesion cavity volume and lessened axonal damage after TBI, but the microglial reaction to the lesion was not affected. We further examined phosphorylation of the microtubule-associated protein tau (MAPT), which aggregates in brains of people with TBI, Alzheimer's disease and Chronic Traumatic Encephalopathy. Surprisingly, *Ccr2* deletion was associated with increased tau mislocalization to the cell body in the cortex and hippocampus by tissue staining and increased protein levels of phosphorylated tau in the hippocampus by Western blot. In conclusion, disruption of CCR2 signaling resulted in enhanced tau pathology and reduced cavity volume in the context of TBI, revealing a dissociation between cavity volume/axonal damage and tau phosphorylation.

T12-31A

Differential balance in STAT1 and STAT3 activation and transcriptional responses to gp130 cytokines in astrocytes versus microglia

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The gp130 cytokines, including IL-6, IL-11, LIF and OSM, have important roles in neuroinflammation and neurodevelopment. However, the nature of any cell-specific responses of astrocytes and microglia to this family of cytokines and their molecular basis is not well understood. Here, the expression of the gp130 family cytokine receptors and subsequent signal pathway activation were examined in murine astrocytes and microglia *in vitro*. Astrocytes had high levels of OSMR mRNA and lower levels of IL-6R, LIFR and IL-11R mRNAs. In contrast, microglia expressed higher levels of IL-6R mRNA, similar levels of LIFR and IL-11R mRNAs, and no detectable OSMR mRNA. In astrocytes, OSM induced STAT1 phosphorylation to a greater extent than hyperIL-6 (IL-6 linked to the soluble IL-6 receptor) and LIF. However, STAT3 activation did not differ significantly between the three cytokine treatments. Conversely, hyperIL-6 and LIF but not OSM, induced phosphorylation of STAT3 and only minimal phosphorylation of STAT1 in microglia. Microarray analysis comparing the response by astrocytes to OSM, hyperIL-6 and LIF revealed up-regulation of many transcripts synonymous with IFN-regulated genes. However, this IFN-like response to OSM was largely abolished in STAT1 KO astrocytes. The gene expression profile of OSM and hyperIL-6 treated astrocytes was somewhat similar while that for LIF differed the most. Despite signalling via a common gp130 pathway, these findings illustrate intercellular and intracellular specificity in the action of the gp130 cytokines in astrocytes and microglia which is achieved through a number of mechanisms that include cell-specific localisation of receptors and differential levels and balance of activation of STAT1 and STAT3.

T12-32A

Functional analysis of TN-C and GFAP induced upregulation in the reactive astrocytes in the injured brain and in primary culture

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Glial fibrillary acidic protein (GFAP) and tenascin-C (TN-C) is well known that prominently upregulated in the reactive astrocytes around injury site in the brain, however the function of these molecules is poorly known.

We have previously reported that Aquaporin 4 (AQP4) is a main water channel exclusively localized to the endofoot of astrocytes in the brain, and its expression is upregulated after the stab wound to mouse brains or the injection of MeHg in common marmosets. Furthermore, glial activation induced by the stab wound injury enhanced by a neuroimmunological function of AQP4 involving osteopontin.

To know the correlation between these molecules in reactive astrocytes, we analyzed the expression level of GFAP and TN-C in stab wound model mouse and in primary cultures using AQP4-deficient mice (AQP4/KO). Immunohistochemistry and Western blot analysis was performed using antibodies against GFAP and TN-C for the brain after the stab wound or the primary culture prepared from wild type (WT) or AQP/KO mice. High levels of GFAP and TN-C expression were observed in activated astrocytes after a stab wound to the brain in WT mice; however, insignificant in AQP4/KO mice. Furthermore, lipopolysaccharide (LPS) stimulation activated the primary culture of astrocytes and upregulated GFAP and TN-C expression in the cells from WT mice, while it was slightly upregulated in the cells from AQP4/KO mice. Moreover, mRNA expression level of inflammatory cytokines was examined in primary culture of astrocytes or microglial cells treated with or without LPS, and found that inflammatory cytokines were upregulated in the cells from WT mice, while modest increases were observed in the cells from AQP4/KO mice. In the absence of a stab wound to the brain or any stimulation of the cells, the expressions of both GFAP and TN-C were lower in astrocytes from AQP4/KO mice than in those from wild-type (WT) mice.

These results suggest that upregulation of GFAP and TN-C in reactive astrocytes induced by stab wound in mouse brain and LPS-stimulated primary culture of astrocytes is dependent on upregulation of AQP4 expression.

T12-33A

CD11c-positive cells from brain, spleen, lung, and liver exhibit site-specific immune phenotypes

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The brain's immune privilege has been attributed to the lack of dendritic cells (DCs) within its parenchyma and the adjacent meninges which implies the maintenance of antigens rather than their presentation in lymphoid organs. Using mice transcribing the green fluorescent protein (GFP) under the promoter of the DC marker CD11c, we identified a intraparenchymal and juxtavascular population of cells expressing CD11c. Since CD11c was also shown to be expressed on other cell types than on DCs, we now phenotypically compared brain derived $CD11c^+/CD45^+$ cells with $CD11c^+/CD45^+$ cells derived from lung, liver and spleen in healthy mice using 7-color flow cytometry. We found unique and site-specific expression patterns of the investigated $CD11c^+$ cells reflecting the common markers F4/80, CD80, CD86, CX3CR1, CCR2, FLT3, CD103 and MHC-II. Moreover, in the brain we observed the two known $CD45^+$ populations ($CD45^{high}$ and $CD45^{int}$), whereas liver, lung and spleen exhibited homogeneous $CD45^{high}$ populations. Most importantly, $CD11c^+$ microglia were unique for their low MHC-II-expression. This was also in line with $CD11c^-$ microglia. In order to test whether phenotypical differences are fixed by origin or develop due to environmental factors, we co-cultivated brain and spleen mononuclear cells on organotypic slice cultures from brain (OHSC) and spleen (OSSC). We show that ramification of $MHC\text{-}II^+$ splenocytes in brain tissue correlates to the down-regulation of MHC-II. In contrast, brain-derived mononuclear cells neither ramified nor up-regulated MHC-II in OSSCs. Thus, brain-derived mononuclear cells maintain their $MHC\text{-}II^-$ phenotype within the environment of an immune organ. Further, our data confirm the view that intraparenchymal $CD11c^+$ cells share established immunophenotypical characteristics of DCs from other organs but remain unique for their low MHC-II expression.

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T12-34A

Sulforaphane inhibits inflammasome activation in murine microglial cells

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Sulforaphane (1-Isothiocyanato-4-methylsulfinylbutane)is a naturally occurring compound in cruciferous vegetables and is organosulfur compound that has isothiocyanate group. Sulforaphane is a phytochemicals which are capable of illustrating anti-inflammatory effects can be good candidate to inhibit inflammasome activation.NLRP3 inflammasome is activated by large number of stimuli like metabolic stress products (cholesterol crystals, ATP, monosodium urate crystals etc.) and exogenous molecules (asbestos, silica etc.) differently rather than the canonical pathways. Pyroptotic cell death may also be caused by inflammasome activation. Therefore, it should be inhibited under such conditions. Main inflammatory glial cell type in the central nervous system (CNS) is microglia. NLRP3 (Nod-like receptor family, pyrin domain containing 3) inflammasome initiates microglial activation which triggers inflammatory responses that IL-1β and IL-18 production controlled by caspase-1 containing multi-protein complex. The view of the present study is to evaluate the effects of sulforaphane on NLRP3 inflammasome activation. We found that sulforaphane decreased the IL-1β cytokine level and cell death caused by LPS-induced inflammasome activation in murine microglial cells.

T12-35A

The role of autophagy in microglial activation

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Autophagy presents one of the major quality control mechanisms of the cell by degrading and removing damaged organelles and various proteins. Loss of the autophagic protein ATG16L1 is associated with Crohn's disease and results in increased proinflammatory cytokine production of macrophages in response to stress (Murthy et al., 2014, Nature 506, 456-462). This finding points to a role of autophagy in the regulation of inflammation. Microglia, the resident immune cells of the central nervous system, are activated in response to infections or cell death resulting in secretion of cytokines. Chronic activation of microglia is hypothesized to contribute to neurodegenerative disorders. To determine a putative function of autophagy in neuroinflammation, we investigated if and how modulation of autophagy would influence cytokine production and secretion of microglia.

Microglia were isolated from the brains of newborn outbreed mice and cultivated with various inducers and inhibitors of autophagy. Their response to activation was determined by analysis of cell morphology and cytokine production. Furthermore, the impact of autophagy modulation on the proinflammatory signaling pathways was investigated. Taken together, our data present a detailed study on the connections between autophagy and microglia activation.

T12-36A

RNA-based regulation of neuroinflammatory responses

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The outcome of an inflammatory response depends on the coordinated regulation of a variety of both pro-inflammatory and anti-inflammatory mediators. And their regulation can occur at multiple levels, including transcription, mRNA translation, post-translational modifications and mRNA degradation. Post-transcriptional regulation has been shown to play an important role in controlling the expression of these mediators, allowing for normal initiation and resolution of the inflammatory responses.

Inflammation-related genes are representative early response genes which have unstable mRNAs due, in part, to the presence of AU-rich elements (AREs) in their 3'-untranslated regions. These AREs provide binding sites for trans-acting factors, such as RNA-binding proteins (RBPs), that can subsequently regulate the stability and/or translation of the mRNA. We also found that some anti-inflammatory small molecules could regulate MAP kinase phosphatase (MKP)-1 expression at the post-transcriptional levels, leading to an attenuation of MAPK signaling, one of the major inflammatory signal pathways. Recently, approaches targeting post-transcriptional control are being under development for drug discovery. Although much remains to be done, RNA-based regulation can be an additional therapeutic strategy for effective control of inflammation.

T12-37A

Interferon-beta induced within the CNS plays a protective role in EAE

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Interferon (IFN)-b is used to treat MS and is effective against experimental autoimmune encephalomyelitis (EAE), an animal model for MS. IFN-b is a member of the type I IFN family, which share a common receptor (IFNAR). The level of IFN-b is increased in the CNS of mice with EAE and lack of type I IFN signalling worsen EAE, suggesting an important role for endogenous IFN-b in the CNS. The aim of this study was to examine the role of CNS-endogenous IFN-b in EAE. Using IFN-b reporter mice we showed that direct administration of poly I:C into the cerebrospinal fluid induced transient up-regulation of IFN-b in CD45/CD11b positive cells located in the meninges and choroid plexus, as well as enhanced IFN-b expression by leukocyte and parenchymal microglial cells. Intrathecal injection of poly I:C to mice at time of onset of EAE substantially increased the normal disease-associated expression of IFN-b and IRF7 in CNS and transiently prevented EAE. This did not occur in IFNAR-deficient mice. Interferon-dependent glial response included production of the chemokine CXCL10. These results show that induction of IFN-b within the CNS can play a protective role in EAE and underscores the role of endogenous type I IFN in mediating neuroprotection.

T12-38A

The role of NG2 in inflammatory disease of the CNS

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The NG2 chondroitin sulfate proteoglycan is a type 1-transmembrane molecule expressed by a variety of cell types throughout the body. It rapidly accumulates at sites of CNS injury and is a prominent component of glial scars, structures considered as main obstacle for efficient neuronal repair. Primary sources of this protein within the CNS are polydendrocytes (NG2 cells), now widely accepted as oligodendocyte precursor cells (OPCs). Using our NG2 reporter/ knockout mouse, we were able to additionally detect this proteoglycan in mural blood vessel cells, as reported by other authors, but not in immune cells or microglia. Furthermore, we have not been able to confirm previous reports that suggested a redundant role of NG2 in experimental autoimmune encephalomyelitis (EAE). To address the cause of this phenotype, we induced EAE in wildtype mice that received bone marrow transplant either from NG2-null or wildtype donors and found no significant difference between these groups. In addition, our preliminary *in vitro* data suggest that proliferation and polarization potential of T helper cells was not affected by NG2 deletion. Further studies, especially those focusing on events occurring at the blood-tissue barriers, are necessary to provide more insights into the role of NG2 in inflammation.

T12-39A

Targeting the CSF-1 receptor alleviates two forms of Charcot-Marie-Tooth disease in mice

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Novel approaches are needed to determine beneficial therapies to treat Charcot-Marie-Tooth (CMT) type 1 neuropathies, which are non-treatable inherited disorders of the peripheral nervous system, causing axonal dysfunction, leading to progressive muscle weakness and a dramatically reduction in quality of life. As primarily genetically-caused diseases, it is not surprising that there is no effective therapy available. However, our group could previously identify low grade, secondary inflammation as a common disease amplifier in distinct CMT1 mouse models. Especially nerve macrophages have a substantial pathogenic impact on disease progression. We now tested a clinical agent targeting macrophages through its inhibition of the colony-stimulating factor-1 receptor (CSF-1R) and demonstrate that in two distinct mouse models of CMT1, the systemic short- and long-term inhibition of the CSF-1R by oral administration leads to a robust decline in nerve macrophage numbers and substantial reduction of the typical histopathological and functional impairment. Our study shows that targeting peripheral nerve macrophages by an orally administered inhibitor of the CSF-1R may offer a highly efficacious and safe treatment option for at least two distinct forms of the presently non-treatable CMT1 neuropathies.

T12-40A

Augmentation of neuropathic pain by DAP12 mediated signal in microglia

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Neuropathic pain due to nerve injury afflicts millions of people. More comprehensive understanding of detailed cellular and molecular mechanisms of neuropathic pain has been sought to establish more effective treatment. Molecular and cellular changes of microglial cells following nerve injury are considered as one of the causes of neuropathic pain. DNAX-activating protein of 12 kDa (DAP12), a transmembrane adaptor protein that contains an immunoreceptor tyrosine-based activation motif (ITAM), is predominantly expressed by microglia in CNS. We previously demonstrated that deficiency of DAP12 in microglia altered fate of nerve injured motor neuron death. In this study we examined an implication of DAP12 in nerve injury induced pain. Following L4 spinal nerve injury, which was an experimental model of neuropathic pain, DAP12 mRNA expressing microglia were increased in the ipsilateral dorsal horn. The real time PCR demonstrated increases of mRNAs for TNF-α, IL-1β and IL-6 in the wild type (WT) mice, however these expression levels were significantly suppressed in the DAP12 deficient (KO) mice at all time points examined from 3 days to 2 weeks after nerve injury. The mechanical threshold measured by the von Frey filaments in the ipsilateral hind paw was significantly attenuated through one to six weeks in the DAP12 KO mice compared with WT mice. Collectively, these results suggest that DAP12 mediated signal may augment neuropathic pain by promoting pro-inflammatory response in microglia. Suppression of DAP12 mediated signal could be a therapeutic target for neuropathic pain.

T12-41A

Local inflammatory cell infiltration in marmosets with experimental autoimmune encephalomyelitis is associated with retinal ganglion cell activation and subpial cortical demyelination

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Introduction: Retinal degeneration and subpial cortical demyelination (SCD) are common in patients with chronic multiple sclerosis. The underlying mechanisms leading to disease pathology in early disease stages remains unclear. Therefore, we investigated the effects of local inflammation accompanied with retinal changes and SCD in marmosets with experimental autoimmune encephalomyelitis (EAE).

Aim: To assess if inflammatory cell infiltration is associated with the activation of retinal ganglion cells and SCD.

Methods: Retina and brain samples from marmosets immunized with myelin oligodendrocyte glycoprotein and control animals were analyzed. To investigate retinal changes, CD3-positive T cells, TUNEL-positive and c-jun-positve cells in the retinal fiber and ganglion cell layer were evaluated. Demyelination was identified by staining for myelin basic protein (MBP) and proteolipid protein (PLP). Meningeal inflammation adjacent to SCD was quantified for CD3-positive T cells, CD20-positive B cells and IgG-positive plasma cells.

Results: In the majority of EAE animals, the retinal analyses showed only single T cells within the retinal fiber and ganglion cell layer, whereas no obvious T cell infiltration was observed in control animals. Density of T cells infiltrating the upper retinal layers was significantly increased in EAE animals compared to controls. Marmosets with retinal T cell infiltration showed increased TUNEL-positive apoptotic cells and c-jun-positive cells within the retinal ganglion cell layer compared to healthy control animals. Meningeal T, B and plasma cells were quantified adjacent to SCD, normal-appearing EAE cortex (NAC) and control marmoset cortex. In marmosets with EAE, meninges adjacent to SCD showed significantly increased T cell and plasma cell numbers, but unaltered B cell numbers compared to NAC.

Conclusion: Local retinal and meningeal T cell infiltration is associated with retinal ganglion cell activation and SCD in marmosets with EAE. These findings indicate that T cells might contribute directly to retinal changes and subpial demyelination in early disease.

T12-42A

Toll-like receptor 3 contributes to inflammatory Schwann cell activation and Wallerian degeneration after peripheral nerve injury

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It is well known that Schwann cells play an important role in Wallerian degeneration after peripheral nerve injury. Recently, it was reported that toll-like receptor (TLR) signaling contributes to Schwann cell activation and Wallerian degeneration. Moreover, previous studies had found that TLR ligands-induced inflammatory Schwann cell is activated via TLR2 and 3. However, the role of TLR3 in Wallerian degeneration after peripheral nerve injury has not been fully elucidated. Hence, the objective of this study is to establish a clearer understanding of the role of TLR3 in Wallerian degeneration after a peripheral nerve injury. It was found that sciatic nerve crush injury reduced the number of degenerating myelin axons in TLR3 knock-out mice. After 7 days, TLR3 knock-out mice showed delayed sciatic nerve degeneration compared with WT mice. In addition, macrophage infiltration into injury site was significantly increased in WT mice, but not in TLR3 knock-out mice. The nerve injury-induced expression of macrophage infiltrated-related chemokines such as CC-chemokine ligand (CCL)2/MCP-1, CCL3/MIP-1a, CCL4/MIP-1b and CCL5/RANTES was compromised in Schwann cells of TLR3 knock-out mice in vitro and in vivo. Similarly, the TLR3 ligands-induced chemokine expression was reduced in Schwann cells derived from TLR3 KO mice. Finally, polyinosinic-polycytidylic acid (poly(I:C)), a synthetic TLR3 agonist, injection into the sciatic nerve of the rat induced macrophage infiltration in vivo. Taken together, these data show that TLR3 is required for the inflammatory Schwann cell activation and contributes to Wallerian degeneration after peripheral nerve injury.

T12-43A

Developmental priming of microglia by n-3 PUFAs deficiency

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Maternal infection during pregnancy is associated with an increased risk for CNS disorders such as memory deficits in the offspring. Such prenatal immune challenge induces inflammatory response in the fetal brain, which may disturb neurodevelopment of the hippocampus in the embryo. The innate immune response in the brain is mediated by microglia, which also play a critical role during neurodevelopment. Their phagocytic activity is involved in removing unnecessary synapses and neurons, allowing effective neuronal connectivity in the mature brain. Because microglia are important players both in neuroinflammation and neurodevelopment, we propose that they are involved in mechanisms by which maternal immune challenge causes brain maturation impairments in the fetus. One preventative strategy to avoid such impairments is to limit the inflammatory response in the fetal brain, which we believe can be achieved through balanced diet. Omega-3 PUFAs have anti-inflammatory properties, and are also important neuronal components. In the present study we investigate whether balancing omega-3 levels in the diet can impact microglia function and prevent neuronal deficits in the mature mouse brain after prenatal immune challenge. To address this question, pregnant females fed with a deficient or balanced diet in omega-3 were injected intraperitoneally with bacterial lipoplysaccharide (LPS), at gestational day 17 and spine density and microglia phagocytic activity were analyzed in the offspring at post-natal days 14 (P14) and 28 (P28). Our data showed that in animals developed under a diet devoid of omega-3, prenatal inflammation leads to a decreased phagocytic activity of microglia regarding exogenous (Quantum dots) and endogenous (PSD-95) elements at P14. We also found that this was paralleled by an increase in dendritic spines and PSD-95 protein levels in the hippocampus at P28. These alterations were not observed in animals developed under a diet balanced in omega-3. Altogether, our data suggest that a prenatal inflammation decreases microglia-dependent synaptic pruning during the maturation phase of the hippocampus, leading to alterations of neuronal morphology later on. All these alterations are rescued by increasing omega-3 content of maternal diet.

T12-44A

IGF1R signaling in oligodendrocytes regulates neuroinflammation without affecting cell survival

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Question: Signaling through the insulin-like growth factor receptor 1 (IGF1R) is critically involved in metabolic control and cell survival. The IGF-1 axis is also a main regulator of differentiation and myelination in oligodendrocyte (ODC) precursor cells. IGF-1 is increased in demyelinating lesions in the CNS of multiple sclerosis patients and has been tested as a neuroprotective factor in numerous inflammatory and neurodegenerative pathologies of the CNS. However, such potential therapeutic

importance is still hindered by the unclear functions of IGF-1 in ODCs, which metabolically and structurally support neuronal functions. In particular, IGF1R signaling during autoimmune demyelination is highly controversial.

Method: To address these questions, we generated mice in which the IGF1R was exclusively deleted in mature ODC. We then subjected this mouse model to experimental insults to the myelin through cuprizone intoxication or induction of neuroinflammation.

Results: Absence of IGF1R from ODCs did not alter their overall density or their myelin status, therefore demonstrating that survival of ODCs in adult mice does not depend on IGF-1 signaling. Moreover, both cuprizone intoxication or induction of neuroinflammation failed to reveal a role of IGF1R in ODC survival or demyelination. Nonetheless, mice deficient for IGF1R on mature ODCs presented with reduced incidence of EAE and overall ameliorated clinical course of disease.

Conclusions: The observed reduction in incidence and severity of EAE highlights the central role of ODCs in the neuroimmunological interplay. IGF-1 signaling in ODCs plays a complex role in the balance between CNS homeostasis and inflammation which goes beyond their mere survival.

T12-45A

Inducible nitric oxide synthase (NOS2) modulation after chronic minocycline in neurpathic pain and influence of selective NOS2 inhibitor on opioid analgesia

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A role of nitric oxide (NO) in the mechanisms of pain generation and transmission throughout the nervous systems has been implicated. Peripheral nerve damage, associated with the glial activation have been associated with the up-regulation of inducible nitric oxide synthase (NOS2) and subsequent release of NO. Selective inhibition of the NOS2 isoform might be a novel approach to manage conditions with neuropathic pain.

The aim of our study was to examine the effect of the microglia inhibitor, minocycline on the expression of NOS2 in spinal cord and dorsal root ganglia (DRG) and effectiveness of selective inhibitor of NOS2, 1400W dihydrochloride on both allodynia and hyperalgesia as well as on opioid-induced analgesia in a neuropathic pain model in rats. The experiments were carried out according to IASP recommendations and local Bioethics Committee. The chronic constriction injury (CCI) of the sciatic nerve was performed. Opioid receptor ligands (morphine and endomorphin-1) and minocycline were injected intrathecally (i.t.) or intraperitoneally (i.p.). Behavioral studies consisted of the allodynia/hyperalgesia measurements while biochemical studies comprised the RT-PCR and Western blot analysis.

A single i.t. administration of 1400W dihydrochloride (30 ug; i.t.) showed significant attenuation of tactile allodynia (von Frey test) and thermal hyperalgesia (cold plate test) at day 7 after CCI. Moreover, 1400W dihydrochloride also strongly potentiated antiallodynic and antihyperalgesic effects of morphine and endomorphin-1. We have demonstrated the strong spinal up-regulation of NOS2 mRNA and protein levels in CCI-exposed rats. Moreover, we have showed a strong up-regulation of NOS2 protein levels in the DRG of CCI-exposed rats. We provide evidence that chronic (twice daily for 7 days) i.p. administration of minocycline (30 mg; i.p.). diminishes the spinal NOS2 mRNA expression and microglial activation. Moreover reduces the NOS2 protein level in spinal cord and DRG. Summing up, our findings confirmed that the administration of 1400W dihydrochloride evoked antiallodynic and antihyperalgesic effects and strongly potentiated antinociceptive effects of morphine and endomorphin-1 in neuropathic pain. Our results prove a role for the NOS2-NO system in the development of pain and the selective inhibition of NOS2 might represent novel, therapeutic target to manage neuropathic pain states. A*cknowledgements:* This study was financed by statutory funds and grant NCN2011/03/B/NZ4/00042.

T12-46A

Novel approaches to image the neuroinflammatory response after stroke by PET

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Introduction: Neuroimaging techniques such as positron emission tomography (PET) and the existence of appealing radiotracers for the translocator protein 18kDa (TSPO), considered as the hallmark for neuroinflammation, have been crucial to characterize the inflammatory response after neurological pathologies such as stroke (Winkeler et al., 2010). Likewise, in vivo imaging of neuroinflammation has been mainly limited so far to the evaluation of this particular receptor in activated microglia. We hypothesize that the *in vivo* PET imaging characterization of novel biomarkers for microglial cells is essential for the establishment of new tools to monitor and modulate neuroinflammation in neurological pathologies such as stroke.

Methods: Longitudinal PET/CT imaging with [^{18}F]DPA-714, [^{11}C]A-836339 and [^{18}F]FSPG was performed to explore the alterations in TSPO, cannabinoid type 2 receptor (CB2R) and cystine/glutamate antiporter (xc^-) binding, respectively, before (day 0) and at 1, 3, 7, 14, 21 and 28 days following transient focal cerebral ischemia in rats.

Results: [^{18}F]DPA-714 showed a progressive increase of TSPO expression during the first week after reperfusion that peaked at day 7, followed by a PET binding decrease from days 14 to 28 in the ischemic hemisphere. In the contralateral territory, [^{18}F]DPA-714 PET showed a significant binding increase at day 7 after ischemia. Likewise, [^{11}C]A-836339 binding evidenced a progressive increase of CB2R expression from day 1 to day 7 followed by a dramatic decrease later on in the infarcted tissue. In the non-ischemic territory, animals experienced a significant increase of the CB2R expression at day 7 in relation to control rats. In contrast, [^{18}F]FSPG showed an early PET signal increase at day 1 followed by the uppermost binding at day 3 and a progressive decline from day 7 to day 14 after ischemia onset. The contralateral hemisphere evidenced a non-significant increase of the xc^- system over the first week after reperfusion.

Conclusions: Altogether these results showed the usefulness of both [^{11}C]A-836339 and [^{18}F]FSPG PET to image novel microglial features on the scenario of cerebral ischemia. Further studies will be conducted to evaluate the therapeutic potential of both CB2R and xc^- system modulation after stroke.

References: Winkeler et al., J Nucl Med. 2010; 51:1-4.

T12-47A

B4GALT6 regulates astrocyte activation during CNS inflammation

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Objective: Astrocytes play complex roles in the response to trauma, infection or inflammation in the central nervous system (CNS). Thus, we wanted to characterize the mechanisms that regulate astrocyte function, and to identify potential targets for the therapeutic modulation of astrocyte activity.

Methods: Non-obese diabetic (NOD) mice develop a disease course that resembles a progressive form of multiple sclerosis (MS), following immunization with MOG. We investigated the role of the astrocytes in promoting pathogenesis, by a lipid-dependent signaling pathway, during the chronic/progressive phase of EAE.

Results: In line with previous studies, depletion of reactive astrocytes during the acute phase of chronic-progressive EAE exacerbated disease. However, depletion of astrocytes in the chronic phase ameliorated the disease. Hence, to understand this dichotomy we compared gene expression profiles

of astrocytes from mice in acute and chronic stages of EAE. One gene associated with the chronic stage was B4GALT6, which codes for the β-1,4-galactosyltransferase 6 that catalyzes the synthesis of lactosylceramide (LacCer).

We observed that LacCer levels are up-regulated in the CNS during chronic EAE, and that LacCer synthesized by the astrocytes acts in an autocrine manner to trigger transcriptional programs that promote the recruitment and activation of CNS-infiltrating monocytes and microglia, and neurodegeneration. We also detected increased *B4GALT6* expression and LacCer levels in CNS MS lesions. Finally, the inhibition of LacCer synthesis suppressed local CNS innate immunity and neurodegeneration in EAE, and interfered with the activation of human astrocytes *in vitro*

Conclusions: Astrocytes promote disease pathogenesis, during the chronic-progressive phase of EAE, by induction of the LacCer-dependent signaling pathway. Thus, B4GALT6 is a potential therapeutic target for MS and other neuroinflammatory disorders

T12-49A

Exploring IFN-ß-mediated new effects on tissue damage prevention in EAE: enhancement of Myeloid-Derived Suppressor Cell immunosuppressive activity

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Multiple sclerosis (MS) is the most frequent autoimmune demyelinating disease of the human central nervous system (CNS). The relapsing-remitting (RR) clinical variant is characterized by phases with increasing neurological symptoms (relapses with myelin destruction) followed by periods of partial recovery (remissions accompanied by myelin repair). This implies that the microenvironment of the demyelinated plaques should be modified by internal or external immunomodulatory agents participating in the promotion of the relapsing-to-remitting transition. Among others, IFN-ß remains to be the most widely prescribed treatment for RRMS, although its participation on myelin preservation or promotion of remyelination are still partially understood. In the last years, our group has been focused on this process, unrevealing the immunosuppressive role of a heterogeneous population of immature myeloid cells, namely the myeloid-derived suppressor cells (MDSCs), during the clinical course of the MS model, experimental autoimmune encephalomyelitis (EAE). Although absent in control conditions, MDSCs (Arg-I^{+}CD11b^{+}Gr-1^{+}-cells) transiently enter the spinal cord of EAE mice showing a parallel density to the severity of the symptoms, and participate in the control of the immune response by accelerating T cell apoptosis. Besides, the undifferentiated state of MDSCs has been linked to their immunosuppressor role, since the polarization of these cells to mature myeloid subsets delays the recovery of the EAE symptoms. Since the main immunomodulatory effects of IFN-ß relies on the reduction of the frequency and severity of the clinical relapses in MS, our group has explored whether they may be mediated by the potentiation of the immunosuppressive role of MDSCs. Our results show that a single injection of IFN-ß decreased the severity of the clinical course of the EAE mice, enriched the presence of MDSCs within the smaller demyelinated areas and preserve the immature phenotypic features of the MDSCs both in the periphery and within the CNS. Moreover, IFN-ß treatment promotes MDSC immunosuppressive function *in vitro* by increasing T cell apoptosis. Taking together, all these data add new insights into the mechanism of the IFN-ß treatment and point to MDSCs as endogenous mediators of its beneficial role in EAE.

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T12-50A

TLR2-induced astrocyte MMP9 activation compromises the blood brain barrier and exacerbates collagenase-induced intracerebral hemorrhage

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The innate immune response plays an important role in the pathogenesis of intracerebral hemorrhage (ICH). Recent studies have shown that Toll-like receptor 2 (TLR2) is involved in the innate immune response in various neurological diseases, yet neither its role in ICH nor the mechanisms by which it functions have yet been elucidated. We examined these in this study using a collagenase-induced mouse ICH model with TLR2 knock-out (KO) mice. TLR2 expression was upregulated in the ipsilateral hemorrhagic tissues of the collagenase-injected mice. Brain injury volume and neurological deficits following ICH were reduced in TLR2 KO mice compared to wild-type (WT) control mice. Heterologous blood-transfer experiments show that TLR2 signaling in brain-resident cells, but not leukocytes, contributes to the injury. In our study to elucidate underlying mechanisms, we found that damage to blood-brain barrier (BBB) integrity following ICH was attenuated in TLR2 KO mice compared to WT mice, which may be due to reduced MMP9 activation in brain astrocytes. The reduced BBB damage accompanies decreased neutrophil infiltration and proinflammatory gene expression in the injured brain parenchyma, which may account for the attenuated brain damage in TLR2 KO mice after ICH. Conclusively, our study revealed TLR2 plays a detrimental role in ICH-induced brain damage by activating MMP9 in astrocytes, compromising BBB, and enhancing neutrophils infiltration and proinflammatory gene expression.

T12-51A

Macrophage activation in perinatal brain injury

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Cerebral palsy is a lifelong disorder characterized by severe motor deficits, and is incurred perinatally by inflammation and hypoxia in the brain. This induces proliferation of oligodendrocyte progenitors yet prevents oligodendrocyte precursor cell survival and differentiation into myelinating oligodendrocytes, causing hypomyelination and impaired nerve function. Current treatments aiming to dampen initial brain injury or manage symptoms are only marginally effective, highlighting the need for therapies that promote myelin repair. Our previous work showed that during efficient myelin repair in the adult brain, microglia and circulation-derived macrophages undergo a switch in activation from a pro-inflammatory phenotype which drives oligodendrocyte progenitor proliferation, to a regenerative phenotype which drives oligodendrocyte precursor survival/ differentiation and myelin repair. We hypothesized that following perinatal brain injury, pro-inflammatory microglia/ macrophage phenotypes predominate over regenerative phenotypes to drive the abovementioned pathology. Our analysis of post-mortem human brain tissue of perinatal brain injury and *in vivo* experimental models of this injury demonstrated an imbalance of microglia/ macrophage activation towards the pro-inflammatory phenotype. We developed a novel *ex vivo* forebrain explant model of perinatal brain injury induced by LPS-mediated sensitization followed by hypoxia, characterized by oligodendrocyte progenitor proliferation, precursor cell death, lack of differentiation and myelination. This was associated with a high ratio of microglia with a pro-inflammatory vs regenerative phenotype. Oligodendrocyte differentiation and/or survival were rescued by supplementation with conditioned media from regenerative microglia or novel factors we identified to be secreted by these cells. Our findings demonstrate that perinatal brain injury modulates microglia/ macrophage activation towards a pro-inflammatory phenotype unable to support oligodendrocyte precursor differentiation and survival, which can be rescued by factors secreted from microglia with a regenerative phenotype. This work has identified microglia and their secreted products as novel therapeutic targets to drive myelin repair following perinatal brain injury.

T12-52A

The role and impact of A20 expression by microglia in neuroinflammation

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Tumor necrosis factor alpha (TNFa) induced protein 3 (TNFAIP3), also known as A20, is a key negative regulator of the canonical NFkB pathway. A20 is an ubiquitin-modifying protein that can act at various levels of the signalling cascade downstream of receptor stimulation to attenuate NFkB activation, thereby regulating the pro-inflammatory response. A number of polymorphisms in the A20 gene have been associated with various autoimmune diseases, including inflammatory bowel disease, rheumatoid arthritis, and multiple sclerosis (MS). Moreover, risk SNPs for MS in the A20 gene correlates with a more severe disease, and A20 expression is reduced in MS patients as compared to healthy individuals. Interestingly, an astrocyte-specific deletion of A20 leads to worsened disease in a mouse model of MS, experimental autoimmune encephalomyelitis (EAE). Furthermore, mice fully deficient for A20 demonstrate spontaneous neuroinflammation, attributed to A20 deletion in either the endothelial cells or the microglia. Microglia are the resident immune cells of the CNS, and usually are in a quiescent state. However, upon insult, microglia rapidly become activated immune-competent cells, producing inflammatory cytokines and chemokines; they also play a role in affecting the integrity of the blood brain barrier. We thus elected to examine the role and influence of A20 expression by microglia in the context of neuroinflammation, by specifically deleting this gene in microglia. For that, we crossed the $A20^{fl/fl}$ to $CX_3CR1\text{-}Cre^{ERT2}$ mice. The $A20^{fl/fl}$;$CX_3CR1\text{-}Cre^{ERT2}$ mice were injected with tamoxifen at two weeks of age, leading to A20 deletion in microglia and many other macrophages. As peripheral macrophages have a limited life span, only microglia remained A20 deficient at adulthood. We found that microglia deficient in A20 spontaneously express significantly higher levels of TNFa mRNA. Surprisingly, we also found significantly more lymphocytes in both the brain and spinal cords of our mice, relative to controls, at steady state. Upon induction of EAE, $A20^{fl/fl}$;$CX_3CR1\text{-}Cre^{ERT2}$ mice develop symptoms sooner than age-matched controls, although the overall disease progression and recovery is similar between the two groups. Under conditions of sub-optimal EAE, however, isolated microglia deficient for A20 were found to express significantly higher levels of MHC Class II and CD40 as compared to controls; we also observe more clusters of activated microglia in the brain when A20 is absent, which often co-localize with MHC Class II expression, as observed by immunohistochemistry. Overall, the deletion of A20 in microglia seems to lead to a spontaneous activation, which may enhance the susceptibility of these animals to developing neuroinflammation under specific conditions.

T12-53A

Neurodegeneration by a microglial complement-phagosome pathway

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Microglial phagocytosis can trigger two distinct signaling pathways. Phagocytosis and killing of pathogens, such as bacteria, are mediated by activation of the nicotinamide adenine dinucleotide phosphate-oxidase (NOX). In contrast, homeostatic phagocytosis of endogenous material is anti-inflammatory, does not activate NOX, and leads to lysosomal acidification to dissolve and digest aggregated material. Recently, we showed by an integrative network-based approach that genes of microglia involved in pathogen phagocytosis with DAP12/TYROBP and NOX as key regulators are highly relevant to neurodegeneration in Alzheimer's disease. We now analyzed an animal model of systemic intraperitoneal application of bacterial lipopolysaccharides (LPS). An elevated microglial inflammatory phenotype with selective neurodegeneration was observed after repeated systemic challenge with LPS. In contrast, application of the same total cumulative LPS dose given once was not leading to neurodegeneration. Whole genome transcriptome analysis of the brains showed an activation pattern involving the classical complement system and its associated phagosomal NOX signaling pathway in the mice repeatedly treated with LPS. Loss of neurons induced by repeated systemic LPS application was rescued in complement C3-deficient mice, confirming the involvement of the complement-phagosome-NOX system in neurodegeneration. Thus, data suggest that the

complement-mediated activation of the phagosomal NOX of microglia is involved in neurodegeneration.

T12-54A

Increased transcripts evidenced in laser-capture microdissected white matter astrocytes during experimental autoimmune encephalomyelitis in relation to immune cell infiltrate

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Astrocytes, the most abundant cell population in the mammal CNS, contribute to a variety of functions including homeostasis, metabolism, synapse formation and myelin maintenance. On the other hand, reactive astrocytes have a deleterious role during autoimmnune demyelination such as multiple sclerosis. In contrast to grey matter astrocyte, the transcriptomic profile of adult white matter (WM) astrocytes is relatively undefined because they cannot be purified *ex vivo*. Here, GFAP-immunolabeled white matter astrocytes were laser-capture microdissected from fresh-frozen spinal cord sections of control female adult mice and of sick mice from experimental autoimmune encephalomyelitis (EAE) using the C57BL6/MOG immunization model. Analysis of RNA obtained from immunolabeled microdissected astrocytes using Agilent microelectrophoresis indicated well preserved RNA. FACS analysis of the rest of each individual EAE spinal cord allowed the determination of immune cell composition showing major infiltration of T cells with an effector/memory CD4+ phenotype in the EAE samples. Using custom Taqman-low-density-array (TLDA), analysis of selected transcript expression from microdissected WM astrocytes of the EAE vs. control mice revealed their reactive signature with EAE-induced gene expression of CCL/CXCL chemokines and of pro-inflammatory molecules described in other CNS disease models. The implication of these observations will be discussed in relation to treatments of neuroinflammatory disorders such as multiple sclerosis. *Supported by Region Pays de la Loire and Fédération de la Recherche sur le Cerveau/Rotary.*

T12-55A

Astrocyte bioenergetics in multiple sclerosis: novel insights to combat neuroinflammation and -degeneration

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Background and aim: The number of mitochondria in demyelinated axons in multiple sclerosis (MS) lesions is enhanced due to an increased energy demand in order to maintain proper conduction. However, chronic inflammation and oxidative stress severely affect the mitochondrial population and evidence is emerging that mitochondrial dysfunction contributes to neurodegeneration in MS. The bioenergetic capacity of neurons critically depends on astrocytes, which provide essential metabolites to neurons. Astrocytes are also involved in the inflammatory response and maintaining the delicate redox balance. The aim of this study was to gain more insight into astroglial bioenergetics in MS and its role in neuroinflammation and -degeneration.

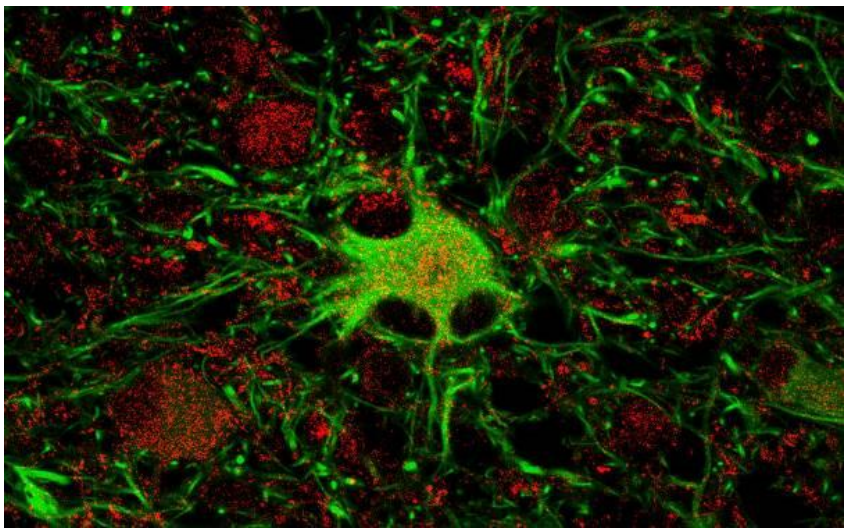
Methods: Immunohistochemistry was used to assess the expression of key proteins involved in glucose metabolism and the metabolic master regulator peroxisome proliferator-activated receptor gamma co-activator 1-alpha (PGC-1α) in MS tissue. PGC-1α overexpressing astrocytes were generated and co-cultured with neuronal cells. ROS and cytokine production and mitochondrial function was assessed using different mitochondrial probes, RT-PCR, ELISA and western blot.

Results: In active MS cases, the expression of rate-limiting glycolysis and citric acid cycle enzymes, as well as key nutrient transporters and PGC-1α, is highly increased in reactive astrocytes. Overexpression of PGC-1α promotes mitochondrial metabolism, enhances antioxidant capacity, but also reduces inflammation and is neuroprotective. In chronic MS lesions the expression of glycolytic,

lactate-producing enzymes and lactate transporters is markedly increased in astrocytes. Demyelinated axons exhibited significantly reduced expression of nutrient transporters, but increased expression of lactate-catabolizing enzymes. Alpha-ketoglutarate dehydrogenase (αKGDH), a key enzyme of the citric acid cycle, was significantly reduced in mitochondria of demyelinated axons.

Conclusions: Our comprehensive immunohistochemical survey of enzymes involved in bioenergetic pathways in MS brain tissue indicates an important role for brain metabolism in neuroinflammation and -degeneration. We demonstrate the neuroprotective potential of astroglial PGC-1α and found evidence for increased astrocyte-axon lactate shuttling in the absence of oligodendrocytes. However, reduced expression of αKGDH and nutrient transporters may hamper axonal energy production thereby contributing to axonal degeneration.

Image



T12-56A

Pharmacological inhibition of CSF1R blocks microglial proliferation and prevents the progression of Alzheimer's-like pathology

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The proliferation and activation of microglial cells is a hallmark of several neurodegenerative conditions. We recently identified the detrimental effects of this proliferative response in an experimental model of chronic neurodegeneration, highlighting the crucial role of the activation of CSF1R in regulating microglial proliferation. In this study, using a transgenic model of Alzheimer's-like pathology (APPswe, PSEN1dE9; APP/PS1) we have studied the temporal and spatial dynamics of microglial proliferation and therapeutically targeted the activation of CSF1R with the specific tyrosine kinase inhibitor GW2580. Our results show progressive proliferation of microglial cells, in close physical interaction with Ab deposition in APP/PS1 mice from 9 to 14 months of age, correlating with the upregulation of the components of the CSF1R pathway. The analysis of post-mortem cortical samples from AD patients evidenced an upregulation of the expression of the CSF1R pathway components, as well as an increased proliferation of microglial cells. Prolonged inhibition of CSF1R in APP/PS1 mice by an orally available drug (GW2580; from 6 to 9 months of age) resulted in the inhibition of microglial proliferation, without affecting microglial survival, down-regulating the expression of the CSF1R pathway and shifting the microglial inflammatory profile to an M2 phenotype.

Specific targeting of CSF1R in APP/PS1 mice caused an improved performance in the open field activity test and spontaneous alternation in the T-maze (short-term memory). The observed improvement of inflammatory and behavioural parameters was not correlated with a decrease in the number of Ab plaques, providing evidence for the uncoupling of the progression of some pathological hallmarks from amyloidosis. Our results provide the first proof of the efficacy of CSF1R inhibition in models of AD, and validate the application of therapeutic strategies aimed at modifying CSF1R activation as a promising approach to tackle microglial activation and the progression of Alzheimer's disease.

T12-57A

Roles of Cx47 and Cx32 in experimental autoimmune encephalomyelitis

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Oligodendrocytes are extensively coupled with other oligodendrocytes and astrocytes by gap junctions (GJs) formed mainly by connexin47 (Cx47) in all oligodendrocytes and by Cx32 in subpopulations of oligodendrocytes. In previous studies we found significant alterations of both oligodendrocyte connexins in the experimental autoimmune encephalomyelitis (EAE) mouse model of multiple sclerosis (MS) as well as in chronic MS brain. In this study we investigated whether the loss of either Cx32 or Cx47 affects the clinical course and pathological changes in mice subjected to EAE. For this purpose, we induced EAE in age-matched (6-8 week old) female fully back-crossed Cx32 knockout (KO) or Cx47KO and C57Bl/6 wild type (WT) mice by MOG_{33-55} peptide immunization. Detailed clinical-behavioral and pathological analysis was performed. We found that EAE induced in backcrossed Cx47 KO mice caused more severe mean clinical score (MCS) on a 5-step scale compared to Cx32KO and WT mice. The MCS of Cx47KO group at the peak of the disease (19-24 days post immunization-dpi) was higher (MCS=3.3 ± 0.4) compared to Cx32KO mice (MCS=2.5 ± 0.2) and to WT mice (MCS=2.0 ± 0.3). Rotarod test of motor performance revealed that the balance and coordination of both Cx32KO and Cx47KO EAE mice was reduced over time compared to WT EAE mice. Furthermore, hind limb grip strength in WT EAE group decreased by approximately 25% of initial strength at 18 dpi, where in both Cx32KO and Cx47KO groups it decreased by 40%. Histological examinations revealed that Cx47KO EAE mice had more demyelination and axonal loss compared to WT and Cx32 KO mice. However, no significant differences in demyelination were observed between Cx32KO and WT EAE mice. Loss of Cx47 also aggravates the disruption of blood spinal cord barrier while the degree of oligodendrocytes apoptosis in EAE mice lucking Cx47 was higher compared to WT and Cx32KO EAE mice. Taken together, these results highlight the importance of Cx47 more than Cx32 in oligodendrocyte survival and hemostasis, and reveal some of the mechanisms leading to demyelination in both genetic and in acquired disorders of oligodendrocyte connexins.

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T12-58A

Therapeutic role of adrenomedullin in Multiple Sclerosis: involvement in remyelination processes

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Introduction: Multiple sclerosis (MS) is a chronic inflammatory, demyelinating and neurodegenerative disease of the CNS. It is characterized by neuroinflammation, autoimmune responses against myelin, oligodendrocyte death and axonal damage that lead to the formation of chronic sclerotic plaques. The pathological loss of myelin is followed by a phenomenon of remyelination, in which oligodendrocytes

synthesize new myelin sheaths to cover the naked axons in CNS. Remyelination occurs in early lesions but fails with disease progression. Current treatments are not curative as are mainly focused on modulating the deregulated immunological response with minor attention to neuroprotective approaches. The best therapeutic agent for MS should combine an immunomodulatory effect with a neuroprotective/regenerative role.

Adrenomedullin (AM) is a neuropeptide produced by CNS and the immune system that has cardiovascular and anti-inflammatory effects. Moreover, AM has shown neuroprotection in experimental brain disease models as ischemic stroke and traumatic brain injury.

Objective: To investigate the potential therapeutic effect of AM targeting the autoimmune and neurodegenerative components of MS.

Methods: We used a pre-clinical model for MS, the experimental autoimmune encephalomyelitis (EAE), that mimics chronic progressive MS, and also a focal model of demyelination by using the toxin lysolecithin. After the daily administration of AM, we analysed clinical and histopathological signs using confocal and electronic microscopy. Also, we performed primary culture for oligodendrocytes isolated from rats in order to study the potential influence of AM to regulate the proliferation, migration and differentiation of these cells.

Results: AM reduced clinical severity and incidence of EAE by decreasing inflammatory infiltration and demyelination in CNS of mice with EAE. Moreover, AM protects oligodendrocytes from oxidative-induced cell death and increase the expression of neurotrophic factors in EAE-treated mice. In addition, the treatment with AM accelerates the remyelination in a focal model of demyelination, by increasing the proliferation and maturation of oligodendrocytes and by reducing astrogliosis.

Conclusions: Our data indicate that AM is a potential candidate as a treatment of MS. The use of AM could represent a therapeutic advantage over current treatments as combines the inhibition of various neuropathological components of the disease, together with an active program of neuroprotection.

T12-59A

Changes of microglia cells associated to aging in a mouse model of accelerated senescence: the SAM P8 mice

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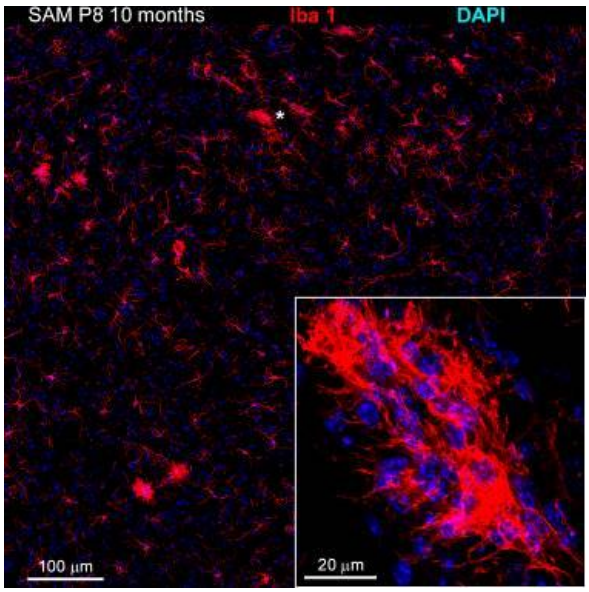
Microglia, the resident brain myeloid cell, constitutes the first line of defense coordinating innate and adaptive immune responses. In healthy mice, microglia has a “quiescent” phenotype but, following acute insults, microglia cells change its morphology, swelling its cytoplasm and shortening their process. This response has been defined as “activated microglia”. Glial activation and inflammatory response factors are features recognized in normal and pathological aging and it has been reported that macrophages and microglia increase in number and change their morphology as well as their cell-surface antigen expression.

We are studying the phenotypical changes of microglia cells associated to aging by using a mouse model of accelerated senescence: the SAM P8 mice and its control mouse strain (SAMR1). We have evaluated microglia changes in these mice with age, studying animals from 2 to 10 months old by using immunofluorescence of Iba 1 (ionized calcium-binding adapter molecule 1) positive cells as microglia marker. Our data show that there are variations in Iba1 positive cells numbers between the different animal backgrounds. Furthermore, we have found an increase in the number of Iba 1 positive cells associated to age in both animal strains: SAMP8 and SAMR1.

We have further evaluated the activated or senescence state of these cells. We have studied the expression of major histocompatibility complex (MHC) class II as marker of activation by immunofluorescence, FACS analyses and x-gal staining immunohistochemistry methods to study

senescence. We have found and increase of MHC-II positive cells with age in meningeal and choroid plexus but not in brain parenchyma of SAMP8 mice. Previous data indicate that the microglia in neurodegenerative diseases is 'primed' rather than activated, and switch their resting phenotype to produce neurotoxic molecules as a response to systemic inflammatory signals. We have used a model of acute inflammation with LPS and analyzed the transcriptional response of isolated adult microglia of SAMP8 and R1 mice in response to this neuroinflammatory insult by analyzing inflammatory genes by real time quantitative PCR analyses. Our data support a primed state of microglia in these strains when compared with C57BL6 mouse strain as control.

Image



T12-60A

Resolvins and lipoxin promote resolution of brain inflammation

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The brain innate immune system is mainly composed of microglial cells. Microglia are activated in response to an immune or inflammatory stimuli or a trauma, and then produce pro- and anti-inflammatory factors. These factors drive the innate immune response and can modulate neuronal activity and *in fine*, learning and memory. Although brain innate immune system defends brain tissue from aggression, chronic activation of microglia can also be deleterious. In the adult brain, chronic production of inflammatory cytokines can contribute to the pathogenesis of neurodegenerative diseases. Limiting the production of pro-inflammatory cytokines and enhancing the production of anti-inflammatory cytokines are crucial for neuron survival. New classes of small and local acting endogenous molecules have recently been identified. Specialized proresolving lipid mediators derived from n-3 polyunsaturated fatty acids (PUFAs), as the resolvins D1 and E1 (RvD1 and RvE1) and from n-6 PUFAs, as the lipoxin A4 (LxA4) are involved in the resolution of inflammation. However their involvement in the resolution of inflammation in microglial cells and the mechanisms by which they influence are unknown. Herein we studied the effects of lipoxin and resolvins on the resolution of inflammation in microglial cells stimulated with lipopolysaccharide. Our results indicated that resolvins and lipoxins were able to inhibit the production of pro-inflammatory cytokines and enhance the production of anti-inflammatory cytokines. Moreover, receptors of LxA4, RvD1 and RvE1 were overexpressed during inflammation, reinforcing the idea that these molecules are involved in the

resolution of inflammation. We also showed that resolvins and lipoxins promoted a phenotypic switch in microglial polarization toward a M2-like phenotype. These findings illustrate novel mechanisms through which PUFAs conferred anti-inflammatory and proresolving actions in inflamed brain.

T12-61A

Functional properties of microglia in mouse models of Alzheimer's disease

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Microglia, resident macrophages of the central nervous system (CNS), survey and support neuronal functions. They participate in oligodendrogenesis and neurogenesis, learning and behavior, and are able to mount crucial innate immune responses upon CNS infection and damage. Ageing and associated neurodegenerative processes may impair these functions. Accordingly, in Alzheimer´s disease (AD), the microglial incapability to clear amyloid β (Aβ) may lead to massive accumulation and deposition of this peptide. On the other hand, microglia seem to be activated in such an environment, leading to excessive production of inflammatory mediators, including cytokines and chemokines, which can further damage the vulnerable CNS circuitry. Here, we investigated properties of microglia isolated from 5XFAD mice at 3 different ages compared to age-matched wild-types (WTs). We cultured the microglia isolated from brains of adult mice and determined their response to the Toll-Like receptors (TLRs) agonists including lipopolysaccharide (LPS) by cyto- and chemokine release and their phagocytotic activity using fluorescently labeled E.coli and myelin. We injected LPS into the brains of 6 and 9 months old mice to study microglia responses *in vivo*, regarding supported recruitment of neutrophils and monocytes from the periphery to the CNS, using flow cytometry analysis.

The current study shows no significant differences between microglia isolated from 5XFAD and WT mice in culture, suggesting that 5XFAD microglia outside of the tissue - and without influences by its environment - can behave like wild-type controls. *In vivo* studies, however, show significant differences between these two genotypes, indicating an impact of the diseased tissue.

T12-62A

Nitric oxide-mediated microglial phagocytosis and why carbon monoxide could be good for the inflamed brain

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Clearance of infected and apoptotic neuronal corpses by microglia during acute inflammatory conditions is an essential process for creating a favorable environment for neuronal regeneration. A characteristic feature of inflammation is the generation of microglia-derived nitric oxide (NO) that plays an essential role in the immune response. Growing evidence indicates that during excessive inflammation activated microglia and the overproduction of NO can elicit neuronal cell death. In contrast, the gaseous messenger carbon monoxide (CO) has been postulated to have neuroprotective and anti-inflammatory properties.

Here, we developed an *in vitro* approach to investigate whether the two gaseous messengers NO and CO influence phagocytic behavior of microglial BV-2 cells during inflammatory conditions. End points of phagocytosis were defined by time-lapse video analysis and confocal immunodetection of neuronal debris within the BV-2 cells. To induce alterations from resting to reactive microglia, we used lipopolysaccharide (LPS) stimulation of cell cultures. LPS-stimulated activation of microglia was accompanied by an increased NO release (Scheiblich et al. 2014). Treatment with LPS or an NO donor enhanced phagocytosis of neuronal debris by resting and reactive microglia (Scheiblich and Bicker 2015). Exposure of microglia to an NO-synthesis inhibitor significantly prevented the LPS-

induced NO release and the phagocytosis of apoptotic neurons. Induction of the CO-generating enzyme heme oxigenase-1 (HO-1) or application of a CO-releasing molecule down-regulated cellular characteristics of reactive microglia, such as enhanced NO production and phagocytic capability. Taken together, our findings reveal a critical role for NO signaling during phagocytic processes and implicate CO as an antagonist to NO in regulating aspects of glial biology. In addition, they support a therapeutic potential of NO synthesis blocking agents and CO-donor based strategies for the treatment of excessive inflammation in the CNS.

Ref: Scheiblich et al. (2014), Brain Res 1564: 9-21; Scheiblich and Bicker (2015), Dev Neurobiol, in press.

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T12-63A

Immune-mediated axono-glial damage - an *in vivo* two-photon imaging approach

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Question: Neuronal damage in autoimmune neuroinflammation is the correlate for long-term disability in patients suffering from Multiple Sclerosis (MS). Here we investigated the processes leading from CNS immune cell infiltration to damage processes at the axon-oligodendroglial unit by intravital imaging of damage mechanisms. We thereby follow up on our recent findings on Th17 cell mediated severe, localized and partially reversible fluctuation in neuronal intracellular Ca^{2+} concentrations.

Methods: We monitored the complex processes of the immune cell attack onto axons and delineated the chain of cause and effect using two-photon laser scanning microscopy of living anaesthetized mice. We employed transgenic mice which express distinct fluorescent molecules in oligocdendrocytes, T cells and report Ca^{2+} dynamics in the neuronal compartment, and which give a functional read-out of these processes. *In vivo* imaging relied on semi-quantitative measurement of neuronal Ca^{2+} fluxes in EAE lesions of living anaesthetized transgenic mice, which carry a Ca^{2+} sensor protein (CertnL15 or TN-XXL mice) and morphologic analysis of myelin damage.

Results and Conclusions: Live-imaging during disease has characterized the neuronal dysfunction as early and potentially reversible. We show here that sustained Ca^{2+} is not necessarily associated with complete demyelination of the axons, but is associated with direct interaction of immune cells with the axono-glial unit.

T12-64A

Modulation of S1P receptors at the Blood Brain Barrier: do astrocytes play an essential role?

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Question: In the central nervous system (CNS) an important structure, the Blood Brain Barrier (BBB), maintains the proper environment to facilitate its functions. The main cellular constituents of the barrier are Endothelial cells (EC), Astrocytes (AC) and pericytes. The interaction between the cell types can improve BBB activity and AC release several factors that increase endothelial barrier properties. In several CNS diseases the BBB can be affected either primarily or as a consequence of the disease, like in Multiple Sclerosis (MS), where a leaky BBB together with demyelinated multifocal lesions are the typical hallmarks. Several pharmacological approach have been developed to modify the course of MS and one of this is the use of the immune modulator Fingolimod, structurally similar to S1P (sphingosine-1 phosphate). S1P is a bioactive sphingolipid that exerts a variety of biological activities,

and both EC and AC express S1P receptors; furthermore, $S1P_1$ and $S1P_3$ are overexpressed in the area of MS lesions. Considering that S1P biology is important for AC and EC, whose interaction and functions are essential for a functional BBB, can S1P receptors modulation modify key BBB properties?

Methods: We used an *in vitro* model in which Human Brain MicroVascular Endothelial Cells and human Astrocytes two adult human immortalized cell lines, were co-cultured allowing us to investigate the effects of S1P on endothelial cells, astrocytes, and interactions between the two . Both ECs and AC were exposed to inflammatory cytokines (TNFα and IFNγ) in the presence of S1P receptors modulators and the effect on cell viability was evaluated. BBB properties were further investigated evaluating the ability of leukocytes to transmigrate through it.

Results: When acting directly on EC, through the stimulation of $S1P_1$, both Fingolimod and S1P are able to prevent EC death caused by the exposure to TNFα and IFNγ. While, acting on AC, S1P receptors modulators induce the release of a factor, GM-CSF, that prevents the effects of cytokines on endothelium. Finally in an *in vitro* BBB model incorporating shear stress, S1P receptor modulation reduces leukocytes migration across the endothelial barrier

Conclusion: The data here reported point out the possibility that Fingolimod, a drug that at the moment is in use in MS for its ability to prevent lymphocytes egress from the lymph nodes, could also act at the BBB reinforcing it and reducing the damage induced by inflammatory condition and the access of leukocytes in the CNS.

T12-65A

Role of STAT3-dependent reactive astrocytes in the spinal dorsal horn in chronic itch

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Chronic itch is an intractable symptom of inflammatory skin diseases, such as atopic and contact dermatitis. Recent studies have revealed neuronal pathways selective for itch, but the mechanisms by which itch turns into a pathological chronic state are poorly understood. Using mouse models of atopic and contact dermatitis, we demonstrate a long-term reactive state of astrocytes in the dorsal horn of the spinal segments corresponding to the itchy skin. Reactive astrogliosis depended on activation of signal transducer and activator of transcription 3 (STAT3). Conditional disruption of astrocytic STAT3 suppressed chronic itch without affecting acute physiological itch, and, notably, pharmacological inhibition of spinal STAT3 ameliorated the fully developed chronic itch. Atopic dermatitis mice exhibited an increase in scratching elicited by intrathecal administration of the itch-inducer gastrin-releasing peptide (GRP), and this enhancement was normalized by suppressing STAT3-mediated reactive astrogliosis. Therefore, our findings indicate that STAT3-dependent reactive astrocytes under chronic itch conditions play as crucial amplifiers of spinal itch signaling, providing a previously unrecognized target for treating chronic itch.

T12-66A

Endothelial protein C receptor expression in microglia is regulated by Sp1

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Question: Microglial are one of the main players of inflammatory responses in central nervous system. They become activated as a result of microbial stimulants and pathological insults. Endothelial protein C receptor is (EPCR) one of the defined receptor of Activated protein C (aPC), which is a

natural anti-coagulant protein with anti-inflammatory and cyto-protective roles. The aim of this project is to examine the role of LPS on EPCR in microglial cells and to unravel the regulation of EPCR pathway.

Methods: In this project, N9 mouse microglial cell line were used. To begin with, we analyzed inductive effects of Lipopolysaccharide (LPS), Peptidoglycan (PGN) and Polyinosinic:polycytidylic acid (Poly I:C) on EPCR expression by qPCR and flow cytometry. Furthermore, we used specific inhibitors to transcription factors that have binding sites in promoter region of EPCR gene, namely Nuclear factor of activated T-cells, cytoplasmic 1 (NFATC1) and Specificity protein 1 (Sp1). We utilized Cyclosporin A (CsA) and mithramycin A (MMA) for inhibition of Nfatc1 and Sp1, respectively.

Results: N9 microglial cells were found to be expressing EPCR mRNA. Moreover, LPS and PGN induced upregulation of EPCR expression and Sp1 transcription factor inhibition suppressed LPS and PGN induced EPCR upregulation.

Conclusions: As a result, our data suggest that LPS and PGN caused up-regulation of EPCR in microglia via Sp1 transcription factor activation.

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T12-67A

Microglia are involved in apoptotic clearance during chronic-relapsing EAE

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Background: Multiple Sclerosis (MS) is a chronic, degenerative disorder characterized by the formation of lesions and infiltration of immune cells in the CNS. Using experimental autoimmune encephalomyelitis (EAE), an animal model for MS, a direct link has been identified between infiltrated immune cells and the progression of lesions as well as clinical symptoms. However, the role of microglia, the innate immune cells of the CNS, is still ambiguous.

Materials & Methods: Chronic relapsing (CR) - EAE was induced in Biozzi ABH mice. This mouse model closely mimics MS with an acute phase, remission and a relapse followed by a secondary progression (chronic phase) of the disease. Microglia were isolated by mechanical dissociation of the spinal cords followed by FACS separation based upon CD11b, CD45 and Ly-6C surface expression. Subsequently, RNA expression levels were analyzed with quantitative RT-PCR. In addition, microglia were examined in EAE spinal cord tissues by immunohistochemistry.

Results: CR-EAE is characterized by extensive infiltration of innate immune cells at the acute phase followed by a fast decrease in numbers at remission that remain low during the chronic phase of EAE. Interestingly, FACS sorted microglia numbers increased at the acute stage and up-regulated MHCII gene expression. This increase in microglia numbers and MHC-II expression was observed throughout the course of CR-EAE. Pro-inflammatory genes were only mildly up-regulated during the acute phase, followed by a return to basal levels at remission. Immunohistochemistry confirmed the increase in the number of microglia and showed that microglia adopt an activated phenotype across the spinal cord. When mice were challenged with LPS for 3 hours an exaggerated response at the gene expression level was detected in microglia during CR-EAE. This was most pronounced at the remission phase, when microglia also up-regulated genes related to the uptake of apoptotic cells.

Discussion: Microglia numbers increase during CR-EAE, but while they respond stronger to inflammatory stimuli, their basal expression indicates that they are not pro-inflammatory. Rather, especially at remission, they up-regulate the expression of apoptotic cell uptake genes. This indicates that microglia are actively involved in CR-EAE, not at the acute phase, but at remission when almost no infiltrates are present in the CNS.

T12-68A

Inhibition of the JNK pathway as a treatment for perinatal diffuse white matter injury

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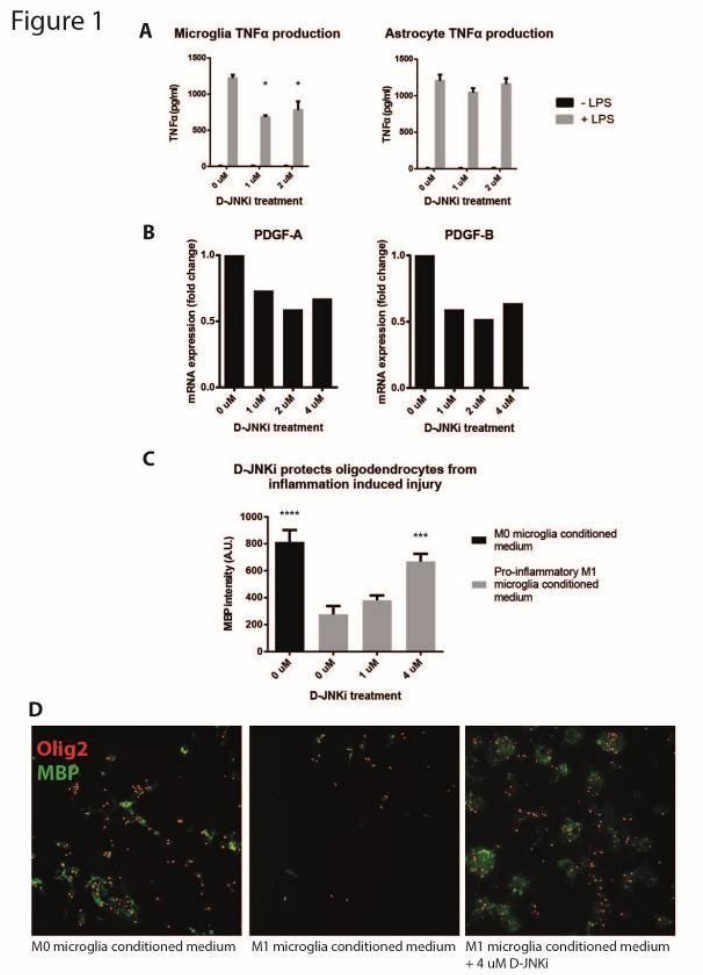
Diffuse white matter injury (WMI) is a neurological disorder commonly observed in neonates born extremely preterm (<28 weeks gestational age). Diffuse WMI has devastating life-long consequences and currently no treatment is available. Interactions between different types of glial cells play an important role in the etiology of diffuse white matter injury. As a consequence of inflammation and disrupted cerebral oxygenation, active microglia and astrocytes create an extracellular environment that is unfavorable for oligodendrocyte precursor cells to fully mature and start proper myelination. This results in altered white matter microstructure causing cognitive, sensory and psychological disabilities later in life.

The negative effects of microglia and astrocytes on oligodendrocytes are mediated by e.g. increased release of proinflammatory cytokines and the production of the differentiation-inhibiting growth factor PDGF. Both the proinflammatory response of microglia and astrocytes, as well as the production of PDGF are mediated by JNK signaling. Furthermore, JNK signaling negatively regulates myelination and mediates apoptosis, for instance by regulating mitochondrial integrity and ROS production as we have shown previously (Nijboer et al., 2013). Using the specific JNK inhibiting peptide D-JNKi, we show *in vitro* that inhibition of JNK attenuates microglial release of proinflammatory cytokines (Figure 1A, left). Astrocytic release of proinflammatory cytokines was not inhibited by D-JNKi (Figure 1A, right). In astrocytes, PDGF mRNA expression was potently reduced after D-JNKi treatment (Figure 1B). Furthermore, JNK inhibition by D-JNKi protects cultured primary oligodendrocytes from inflammation-induced injury (Figures 1C and 1D). To summarize, we show that D-JNKi can have positive effects on oligodendrocytes directly, but also indirectly by mitigating the negative effects of microglia and astrocytes on oligodendrocyte maturation. Our results indicate that inhibition of the JNK pathway is a promising therapeutic strategy to treat perinatal diffuse WMI.

Image

Figure 1



T12-69A

The phenotypes of microglia and macrophages during epileptogenesis

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Question: A growing body of evidence is now supporting a relationship between inflammation and epilepsy. Indeed, activated microglia, reactive astrocytes, local expression of pro-inflammatory cytokines, blood brain barrier leakage and peripheral immune cell infiltration have all been observed in temporal lobe epilepsy (TLE) animal models as well as in humans. Accordingly, inflammatory mechanisms are thought to play a central role in the initiation and maintenance of seizures, starting in the acute phase represented by status epilepticus (SE) induction. Microglia activation has been correlated with the expression of several pro-inflammatory cytokines which are thought to contribute to the neuronal cell death occurring after SE. Data point towards a pro-inflammatory phenotype of microglia that precedes neuronal injury and cell death. Because of this, microglia are generally considered to play a pro-epileptogenic role. However, infiltration of peripheral immune cells during epileptogenesis such as leukocytes, granulocytes and monocytes/macrophages might also contribute to the development of chronic epilepsy and recurrent seizures. Uncertainty on the role of these

different inflammatory cells depended on technical limitations in the discrimination of microglia from macrophages. For this reason, it is possible that the detrimental function that is currently attributed to microglia might be incorrect and should be ascribed to infiltrating macrophages.

Methods: Both microglia and macrophages were acutely isolated from the hippocampi of control and pilocarpine-treated CD1 mice (24h and 96h after SE) and FACS sorted. Microglia were defined as $CD11b^{+}$ $CD45^{int}$ Ly-$6C^{neg}$ and infiltrated macrophages as $CD11b^{+}$ $CD45^{hi}$ Ly-$6C^{pos}$. After sorting, qPCR and flow cytometry analysis were performed.

Results: During epileptogenesis, microglia displayed a weakly immune-activated phenotype, based on the expression of MHCII, co-stimulatory molecule CD40 and pro-inflammatory gene IL-1β. In contrast, infiltrated macrophages were strongly immune activated. Both cell types expressed high levels of the phagocytosis marker AXL.

Conclusions: These data suggest that macrophages might be more detrimental than microglia during epileptogenesis.

T12-70A

A mouse model of atopic diathesis displaying tactile allodynia with glial inflammation in the spinal cord

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Objective: To elucidate the mechanisms underlying tactile allodynia in patients with atopic myelitis using a mouse model of asthma.

Methods: Six-week-old male C57BL/6 mice were used in this study. To establish the model, 50 μg ovalbumin and 2 mg of aluminum hydroxide was dissolved in 200 μl phosphate buffer and intraperitoneally (i.p.) injected once a week. In the third and sixth weeks, mice inhaled 2.5 mg/ml ovalbumin for 5 consecutive days to induce bronchial asthma. At the end of the third and sixth weeks, tactile allodynia was evaluated using stimulation with von Frey filaments, and animals were sacrificed for immunohistochemical analysis. Microglia were collected from these mice and used for gene expression analysis. Peripheral blood samples were also collected for the evaluation of cytokine levels in each group. Some mice were treated with 30 mg/kg/day minocycline (i.p.) for 7 days before the induction of bronchial asthma.

Results: Tactile allodynia was observed in the mouse model of bronchial asthma, but not in the PBS control group. In the spinal cord, microglial activation was seen mainly in the dorsal horn region. Microglia were more numerous and ramification was reduced. Astrocytes were also activated and increased in number. Endothelial cell activation was present, and the integrity of the blood-brain barrier was compromised. RNA array analysis of microglia revealed activation of several pathways, including the ubiquitin-mediated proteolytic pathway, as well as cell cycle and endocytotic pathways. We also observed an increase in expression of endothelin receptor type B (Ednrb). Immunohistochemical analysis showed increased expression of endothelin-1 (ET-1), the ligand for EDNRB, in serum, and the main source of EDNRB in the parenchyma was astrocytes. Minocycline treatment successfully alleviated the allodynia with suppression of glial inflammation. Furthermore, we found elevation of ET-1 levels in atopic myelitis patient sera.

Conclusion: Microglial activation is present in the mouse allergic asthma model. These mice develop tactile allodynia, which simulates the neuralgic pain/dysesthesia observed in patients with atopic myelitis. The elevation of ET-1 levels in the serum of these mice and in patients with atopic myelitis suggests at least partially similar pathogenetics in the murine model and in the human disease. Furthermore, our findings suggest that minocycline may have therapeutic potential in the treatment of tactile allodynia in patients with atopic diathesis.

T12-71A

TUDCA skews microglia towards M2 phenotype through the G-protein coupled bile acid receptor GPBAR1/TGR5

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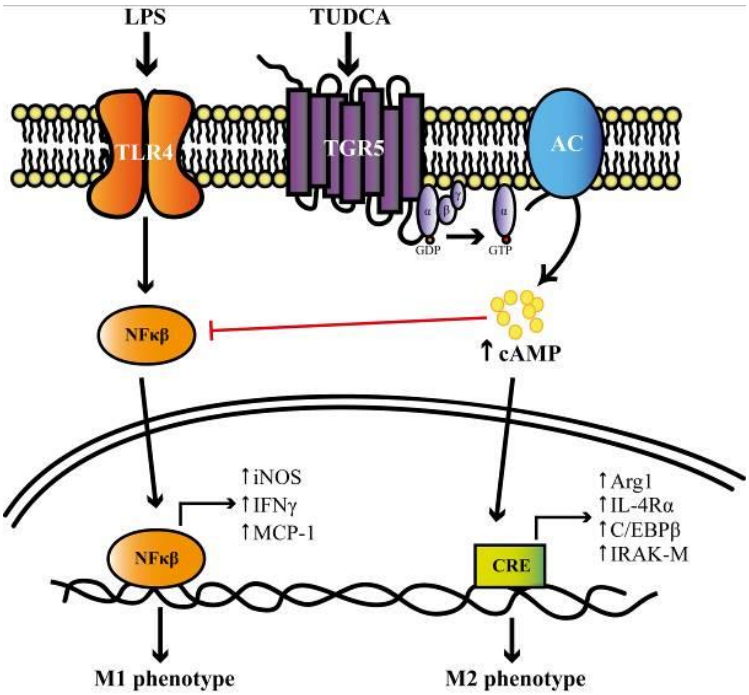
Tauroursodeoxycholic acid (TUDCA) is a bile acid conjugate with neuroprotective and anti-inflammatory effects in several neurological diseases. Previous work from our lab has shown that TUDCA has an anti-inflammatory effect on both, glial cell cultures and on an animal model of acute neuroinflammation (intracerebroventricular injection of bacterial lipopolysaccharide (LPS,) in C57BL/6 adult mice. TUDCA administration reduced glial cell activation, limited microglial cell migration and decreased the expression of the chemoattractants (e.g., MCP-1) and vascular adhesion proteins (e.g., VCAM-1) required for microglial migration and blood monocyte migration to the CNS inflammation site. Moreover, TUDCA polarized microglia towards a M2 anti-inflammatory phenotype, inducing M2 markers, such as Arg1 or IL-4Rα.

Here, we show that the G-protein coupled bile acid receptor GPBAR1/TGR5 was expressed *in vitro* and *in vivo* on the surface of microglial cells and it was responsible for TUDCA-induced anti-inflammatory effect, by increasing cAMP levels. Reduction of GPBAR1/TGR5 expression with specific siRNAs or by inhibiting PKA activity with Rp-cAMPS, prevented TUDCA-induced anti-inflammatory effect (reducing NFκβ activation by LPS) and suppressed the induction of M2 microglial markers.

We propose that TUDCA would be a beneficial therapy for CNS pathologies that present inflammation.

This work was supported by grants from the FISCAM-Servicio de Salud Castilla-La Mancha (SESCAM, PI2008/19 and PI2009/51), and the Plan Nacional from the Spanish Ministry of Economy and Competitivity (SAF2009-11257 and SAF2012-40126).

Image



T12-01B

Role of glial cells in the neuroinflammatory damage induced by ethanol through TLRs/NLRs receptors

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The innate immunity takes a principal function based on recognize microbial structures that are present in pathogens. Toll-like receptors (TLRs) and nucleotide-binding oligomerization domain (NOD)-like receptors (NLRs) are the major pattern recognition receptors (PRRs) that regulates the innate immune response against pathogenic or injury conditions. Our results indicated that ethanol-induced TLR4 activation triggers signalling inflammatory responses in glial cells, causing neuroinflammation and brain damage. However, it is uncertain if ethanol is able to activate NLRs/inflammasome in the glial cells or neurons, the underlying mechanisms involved, and whether there is crosstalk between both immune sensors in CNS. Here, we show that chronic ethanol treatment increases the co-localization of caspase-1 in both, glial and neuronal cells, up-regulates the release of IL-18, IL-1β, IFN-γ and IL-33 cytokines and the production of several neuroinflammatory chemokines (MCP-1/CCL2, CCL3, CCL4, etc.) in the frontal medial cortex from WT, but not in TLR4 knockout mice. Our in vitro studies indicates that astroglial and neuronal cells, both expressed several caspase-1 dependent inflammasomes, although NLRP3 mRNA is the predominant form. We show the effects of different treatments with ethanol, ATP or LPS are able to up-regulates NLRP3 expression, and causes caspase-1 cleavage and the release of IL-1β and IL-18. Moreover, we demonstrate by the first time, how mitochondrial (m) reactive oxygen species (ROS) generation mediates in ethanol-induced NLRP3/caspase-1 inflammasome activation, because using specific inhibitors, we are able to abrogate mROS release and reduce the up-regulation of IL-1β and IL-18 induced by ethanol or LPS or ATP. We further confirm by confocal microscopy studies that ethanol, ATP or LPS promotes NLRP3/caspase-1 complex recruitment within the mitochondria to promote cellular death by caspase-1-mediated pyroptosis. Suppression of the TLR4 function abrogates most ethanol effects on NLRP3 activation and decreases cell injury. These results suggest that NLRP3 participates in the alcohol-induced neuroinflammation through TLR4 activation signalling pathway. These findings highlight the role of TLR4/NLRP3 crosstalk in alcohol-induced brain injury and suggest new therapeutic targets in the effects of ethanol on the brain. (S*upported by RED-RT: RD12-0028-007and SAF2012-33747)*

Image

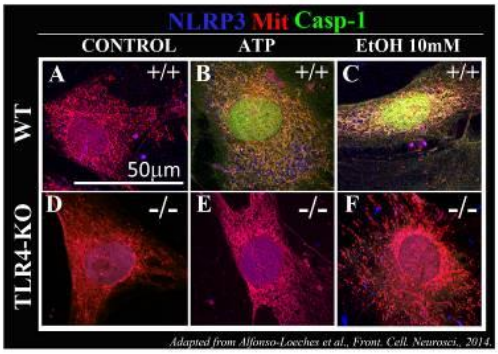

Figure 1: Confocal images of NLRP3/caspase-1 co-localization within mitochondria in treated/non-treated astroglial cells with ethanol and ATP. The microphotographs show that the ATP (5mM) or ethanol (10mM) treatments promote the co-localization of NLRP3 inflammasome (blue) with the active caspase-1 (Casp-1, green) within mitochondria (Mit, red) in the WT-astrocytes when compared to untreated control astrocytes (A-C). No significative changes were observed for treated and non-treated TLR4-KO astrocytes (D-F).

Figure 1

T12-02B

Neuropeptide Y Y_1 receptor modulates microglia activation in the rat retina

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Increasing evidence has shown that Neuropeptide Y (NPY) is a regulator of inflammatory responses. In response to retinal injury, microglia become activated, resulting in increased levels of pro-inflammatory cytokines, increased inducible nitric oxide synthase (iNOS) expression and NO production, which can lead to neural degeneration. In the retina, microglia activation has been associated to a number of different pathological states including diabetic retinopathy and glaucoma.

The aim of this work was to investigate whether NPY, particularly through Y_1 receptor subtype activation, is able to inhibit retinal pro-inflammatory responses mediated by microglia.

The expression of NPY and NPY receptors was assessed in retinal microglia by immunofluorescence in cultured rat retinal explants and purified cultures of retinal microglial cells. In retinal explants, an inflammatory response was induced by exposure to lipopolysaccharide (LPS), in the absence or presence of NPY, Y_1/Y_5 receptor agonist ([Leu^{31}, Pro^{34}]-NPY; LP-NPY) and/or Y_1 receptor antagonist (BIBP3226). TNF, IL-1beta and IL-6 levels were assessed by ELISA. An animal model of retinal ischemia-reperfusion (I-R) injury was used. NPY was injected intravitreally 1h before ischemia (1 h duration) and the inflammatory responses were evaluated after 8 h or 24 h reperfusion.

Immunoreactivity of NPY and Y_1, Y_2 and Y_5 receptors was detected in retinal microglia. In cultured retinal explants, NPY and LP-NPY prevented LPS-induced morphological changes in microglial cells and BIBP3226 abrogated the effect of LP-NPY. Pre-treatment with NPY and LP-NPY also inhibited the LPS-induced increase in iNOS immunoreactivity in microglial cells and BIBP3226 abolished the effect of LP-NPY. Exposure to LPS increased TNF, IL-1beta and IL-6 levels in retinal explants. Pre-treatment with NPY inhibited IL-1beta and IL-6 increase, but not TNF, whereas LP-NPY significantly inhibited TNF, IL-1beta and IL-6 increase. BIBP3226 abrogated the effect of LP-NPY.

Intravitreally NPY administration prior ischemia inhibited TNF, IL-1beta and IL-6 increase induced by I-R injury and the percentage of amoeboid microglial cells in the retina.

Our results show that NPY and Y_1 receptor activation exert anti-inflammatory effects in the retina, by inhibiting microglia activation triggered by LPS and I-R injury.

FCT (Grant PTDC/NEU-OSD/1113/2012 and Strategic Project PEst-C/SAU/UI3282/2011-2013, UID/NEU/04539/2013), Portugal, COMPETE-FEDER, and AIBILI.

T12-03B

Differential inflammasome expression and activation in glial cells

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IL-1β production has been linked to neuroinflammatory conditions and neurodegenerative diseases for a long time with both beneficial and detrimental effects described. However the potential role of the NLRP3 inflammasome in the local production of IL-1β in the brain is not very well understood. Recently several reports started to investigate inflammasome activation in neuroinflammatory and neurodegenerative conditions, in spite of this fact a lot of basic questions remain. We therefore wanted to characterize in more detail the *in vitro* capacities of microglia and astrocytes separately to respond to inflammasome activation, since this issue has not clearly been addressed. Our preliminary results suggest that the capacity to form a functional NLRP3 inflammasome is limited to the microglial compartment in the brain. Microglia are able to produce IL-1β in response to different classical inflammasome activators, such as ATP, nigericin or MSU. We were not able to observe any IL-1β expression and/or secretion from astrocytes, at least not in the conditions tested. Nevertheless, astrocytes expressed several other inflammasome components, such as NLRP1, AIM2, upregulation of caspase-11 and IL-18 mRNA, suggesting that astrocytes could form alternative inflammasome complexes.

T12-04B

IL4 exposure broadly represses TLR-induced cytokine responses in primary microglia

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Interleukin (IL) 4 is a cytokine that affects both adaptive and innate immune responses. In the central nervous system, microglia express IL4 receptors and it has been described that IL4-exposed microglia acquire anti-inflammatory properties. However, the effects on innate immune responses are less well characterized. We here demonstrate that IL4 exposure induces changes in the microglial surface protein expression profile and enhances their potential to induce proliferation of T cells with a regulatory signature. More importantly, we show that TLR-induced cytokine production is broadly impaired in IL4-exposed microglia at the transcriptional level. We demonstrate that IL4 type 2 receptor-mediated signaling is crucial for the inhibition of microglial innate immune responses. In addition, we wil show data on the identification of the molecular mechanisms that underlie the inhibition of TLR-induced responses in IL4-exposed microglia which may aid the design of strategies that aim to modulate innate immune responses in the brain.

T12-05B

Phagocytic gliapses precede cellular elimination leading to targeted phagoptosis in the brain

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Microglial cells are able to phagocytose entire cells in healthy and pathological circumstances of the brain. However, it remains unclear whether microglia phagocytose just as scavengers or whether the process of phagocytosis requires specific cell death induction or phagoptosis. In the present work we show evidences that microglial cells are able to phagocyte entire cells in different scenarios of brain damage *in vivo*. This phagocytosis is preceded by the apposition of microglial cells to the target, forming the so-called phagocytic gliapse, which is characterized by the formation of a flat interface, accompanied by the polarization of organelles towards the intercellular space. In this process Rho/Cdc42 signaling is fundamental for the specific polarization of the cells, as well as the polarization of Iba-1 and F-actin in the conformation of lamellas and leading edges. The polarization of the Golgi apparatus to the interface and the one-to-one gliaptic-target suggest that gliaptic establishment may be critical in phagocytosis-dependent cell death induction in brain pathology.

This work was supported through grants from the Spanish Ministry of Economy and Competitiveness (RYC2010-06729, SAF2010-21274 and SAF2013-45178-P).

T12-06B

Antibody and complement-mediated glial response and demyelination

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Multiple Sclerosis (MS) is a CNS disease driven by inflammatory and neurodegenerative components. Although MS is thought to be a T cell-mediated disease, many MS lesions show deposition of immunoglobulin and activated complement (C). Detection of oligoclonal bands in cerebrospinal fluid, a sign of intrathecally-synthesized IgG, is characteristic of MS. B cells and antibody-producing plasma cells are also present in the meninges and perivascular space. Myelin oligodendrocyte glycoprotein (MOG) is a candidate autoantigen in MS . Injection of anti-MOG into rats with pre-established experimental allergic encephalomyelitis (EAE) increased disease severity and demyelination. The related disease Neuromyelitis Optica (NMO) is known to be mediated by antibodies, and pathology can be transferred to mice by intrathecal injection of NMO IgG + C. Mice lacking the C regulator CD59a develop more severe demyelination and axonal damage in NMO and EAE. This indicates that C regulation plays an important role in controlling CNS pathology. We hypothesized that anti-MOG antibodies could induce C-dependent demyelinating pathology. In this study, we investigated the role of C and antibodies in CNS demyelination.

Injection of anti-MOG Mab with C into corpus callosum of C57BL/6 mice induced demyelination after 2 days. Demyelination was enhanced by CD59a blockade using a specific Mab. In the absence of exogenous complement, but with CD59a blockade, demyelination was still detectable though reduced. Control Mabs did not induce demyelination. Antibody-mediated damage was accompanied by an inflammatory response involving activation of astrocytes and microglia. Astrocyte response (shown by staining with anti-GFAP) was C-dependent, varied with dose of anti-MOG, and was not enhanced by CD59a blockade. By contrast, microglial response (shown by staining with anti-Iba1) was independent of dose of anti-MOG, and was also induced by anti-CD59a + C. Studies are ongoing to determine whether CD59a expression by microglia plays any role in their response. Other anti-myelin as well as anti-axonal specificities are also being examined

The finding that blocking the endogenous C regulator CD59a enhances antibody-mediated demyelination points to a role for C in CNS pathology as well as raises questions about source of C in the CNS. This has relevance to understanding pathogenic mechanisms in MS.

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T12-07B

Intracerebroventricular insulin presents different neuroinflammatory effects in young and aged hippocampus of Wistar rats

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Insulin signaling has been described as a key pathway responsible for learning and memory in the hippocampus. Insulin resistance has been reported to occur in the aging brain. The mechanisms underlying neurodegeneration associated with cognitive decline in aging brain involve brain insulin resistance and persistent neuroinflammation. Up to now, it is not known if brain insulin signaling mediates neuroinflammatory responses in both young and aging brain. To investigate this unknown field our main question was how is the neuroinflammatory response of young and aged hippocampus to insulin treatment, and if this response results in an improved spatial memory formation. For this we used young (4 months) and aged (22 months) Wistar rats that were intracerebroventricular (i.c.v.) injected with insulin (20 mU) or vehicle (saline) for five consecutive days. One group of animals was sacrificed and submitted to inflammatory profile tests, while the other group was subjected to Morris Water Maze task to assess spatial memory. The neuroinflammatory profile was evaluated by immunohistochemistry (Iba-1/ED1 staining) to evaluate microglial cell counts and activation in the three main hippocampal sub regions CA1, CA3, DG; western blot analysis of COX-2/p-nfk-β/nfk-β and luminex analysis of hippocampus to detect the interleukins GM-CSF, IL-1β, IL-6, IL-10, IL-2 and IL-12. Our data showed that the microglial cells in aged hippocampus were reduced in number but more activated compared to young hippocampus. Insulin increased the number of microglia in CA1 and CA3 and microglial activation in all sub regions of young hippocampus but not in aged hippocampus (Fig.1 A-G). Our results also indicated that insulin increased COX-2 expression and decreased nfK-β phosphorylation in young animals and these markers were not affected in aged animals (Fig. H;I). Interleukins analysis showed that insulin increased GM-CSF and IL-12 only in young animals and IL-10 only in aged animals, but increased IL-1β, IL-6 and IL-2 in both young and aged animals (Table1). Insulin treatment improved spatial memory only in young animals, but not in aged rats (Fig.1 J;K). These results suggest that there probably is neuroinflammatory insulin signaling in the hippocampus that contributes to spatial memory processing in young hippocampus, but not in aged one. The different neuroinflammatory profile and spatial memory behavioral viewed in aged animals would to be linked with the insulin signaling disruption, since these animals were challenged with the same insulin treatment as young animals.

Image

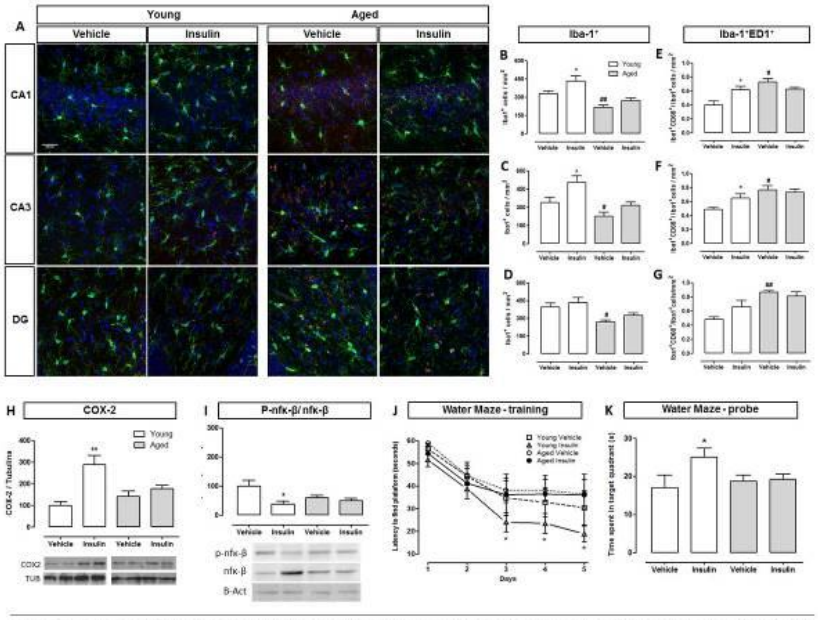

Figure 1: Insulin presents different neuroinflammatory effects and spatial memory performance in young and aged hippocampus. A) Representative images of Iba-1 (green), ED1 (CD68) (red) and DAPI (blue) markers in CA1, CA3 and DG hippocampal sub regions. B;C;D) Quantification of microglial number by Iba-1+ cells/mm2 in CA1(B), CA3(C) and DG(D) hippocampal sub regions. E;F;G) Quantification of microglial activation by cells presenting Iba-1+ED1+ colocalization count in CA1(E), CA3(F) and GD(G) hippocampal sub regions. H) Representative images and quantification of COX-2 expression in homogenized of young and aged hippocampus. I) Representative images and quantification of nfκ-β phosporilation in homogenized of young and aged hippocampus. J;K) Spatial memory performance by Morris Water Maze task; Latency (s) to find the plataform in the training phase during 5 days (J) and time (s) spent in target quadrant without the plataform in the probe phase one day after the fifth training day (K). CA1=Cornus Amonis 1, CA3=Cornus Amonis 3, DG= Dentate Gyrus (Results = media±SEM; */#= p<0.05; **/##=p<0.01)

Table

Interleukins pg/mL/mg protein	Young Vehicle	Young Insulin	Aged Vehicle	Aged Insulin
GM-CSF	1.83±0.418	4.0*±0.581	1.73±0.619	1.87±0.5
IL-1β	13.07±2.5	32.05*±10.686	8.89±3.272	35.22*±12.596
IL-6	1.9±0.528	3.90*±1.555	-	1.76*±1.168
IL-10	65.31±19.164	86.27±20.79	23.52±7.0	88.55*±23.632
IL-2	2.8±2.1	39.30*±3.524	-	8.60*±5.5
IL-12	3.01±0.96	54.65*±24.945	-	1.63±0.395

Table 1: **Insulin affects interleukins expression in young and aged hippocampus.** Luminex analisys of hippocampal homogenized interleukins expression in young and aged animals treated with i.c.v. insulin or vehicle. (Results = media±SEM; *= p<0.05)

T12-08B

Inflammasome-induced IL-1β secretion in microglia is characterized by delayed kinetics and is only partially dependent on inflammatory caspases

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Activated microglia are a prominent hallmark of neuroinflammatory and neurodegerenative diseases. Microglia can be activated via innate immune receptors by pathogens or by more general cellular stress, for example caused by neuronal degeneration. Recent studies have implicated NOD-like receptor (NLR)-mediated activation of microglia in several neurodegenerative and infectious brain diseases. Upon activation, NLR can form multiprotein complexes with inflammatory caspases, called inflammasomes. Inflammasomes link pathogen recognition and cellular stress to the processing of the pro-inflammatory cytokine interleukin (IL)-1β. Whereas inflammasome-mediated activation is heavily

studied in hematopoietic macrophages, much less is known about microglia. Although microglia resemble hematopoietic macrophages in phenotype and function, they originate from a distinct progenitor. To directly compare inflammasome-mediated activation in different types of macrophages, we isolated primary microglia and hematopoietic macrophages from adult, healthy rhesus macaques. We analyzed the expression profile of NLR and inflammatory caspases and characterized inflammasome activation and regulation in detail. We here demonstrate that primary microglia can respond to the same innate stimuli as hematopoietic macrophages. However, microglial responses are more persistent due to lack of negative regulation on pro-IL-1β expression. In addition, we show that while caspase 1, 4 and 5 activation is pivotal for inflammasome-induced IL-1β secretion by hematopoietic macrophages, microglial secretion of IL-1β is only partially dependent on these inflammatory caspases. These results identify key cell type-specific differences that may aid the development of strategies to modulate innate immune responses in the brain.

T12-09B

Astrocytes as a key partner in Methamphetamine-induced Microglia activation

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Methamphetamine (Meth) is a highly addictive psychostimulant posing severe health consequences and long-term neurotoxic effects. Microglial cells, the resident immune cells of the central nervous system (CNS), play crucial roles in inflammatory processes. Upon activation, microglia secrete an array of molecules including pro-inflammatory cytokines, NO and ROS, which can cause damage to the neuronal cells. Of note, microglia activation was reported in Meth users. Recent *in vivo* and *in vitro* studies pointed microglia as a possible major mediator of Meth-induced neurotoxicity. In this study, we investigated the role of Meth in microglia and astrocytes activation, as well as the crosstalk between them. To gain further insight into microglia activation we analyzed the effects of Meth exposure in pro- and anti-inflammatory markers, using primary cortical microglia cultures, immunocytochemistry, quantitative fluorescence microscopy and qRT-PCR. We show that Meth did not trigger a pro-inflammatory or an anti-inflammatory signature in microglia. We also evaluated the Meth effects on primary astrocyte cultures using reactivity markers (f-actin, GFAP and iNOS) and observed that Meth did not trigger astrocyte activation. Moreover, in an attempt to clarify how Meth could affect the crosstalk between microglial cells and astrocytes, we evaluated the effect of conditioned media from astrocytes (ACM) treated with Meth in microglial cultures. We found that ACM treated with Meth induces a pro-inflammatory signature significantly increasing ROS production, iNOS expression and the phagocytic activity, when compared to cells incubated with conditioned media from naïve astrocytes. In order to isolate possible astrocyte-released factors under Meth exposure, we analyzed the production of pro-inflammatory cytokines (IL-1B; IL-6 and TNF) by qRT-PCR and glutamate release by time-lapse video microscopy coupled with FRET-based high sensitive biosensors. We observed no change in pro-inflammatory cytokine production and a huge increase in glutamate release from astrocytes in presence of Meth.
Contrarily to the commonly believed, here we describe that Meth *per se* triggers neither cortical microglia nor astrocytes activation. Nonetheless, Meth-induced microglia activation appears to be mediated by soluble factors released from Meth-sensitized astrocytes, suggesting that Meth induces microglial activation in an astrocyte-dependent manner and a possible mediator for this crosstalk is the glutamate.

T12-10B

Microglial Microvescicles as therapeutic vector for neuroinflammation

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Extracellular vesicles EVs are membrane-bound particles formed from inside a cell or directly from its membrane and released to the extracellular space that carry information whose function is cell-to-cell communication without direct contact. EVs can be divided by their biogenesis, cell origin and morphologic characteristics, in to three classes: exosomes, microvescicles (MVs) and apoptotic blebs.

Most of cells release EVs; MVs may represent a very promising strategy to gain pathogenic information, and identify therapeutic targets. MVs may act as “physiological cargo” and with their low immunogenicity can be engineered as potential therapy delivery systems. Recent evidence suggest that activated microglia uses EVs to communicate to neighbouring microglia and modulate their phenotype and function. We propose here to exploit microglia-derived EVs as drug delivery tool for neuroinflammation and neurodegeneration, trough production of microglial MVs able to cross the blood brain barrier (BBB) and deliver therapeutic molecules to the central nervous system (CNS). We produced a stably engineered murine microglia cell line to express interleukin 4 and Rabies viral glycoprotein RVG. We use IL-4 because it can shift microglia to a protective phenotype called M2 and the RVG has a high affinity to the isoform α7 achetil-coline receptor that is expressed on the surface of neurons, astrocyte, microglia and other cells. The capacity of RVG to target the CNS is already demonstrated. We optimized the microglial MVs production using ATP and PMA and the collection by different step of centrifugation. We evaluated if the MVs can transfer their content in vitro using farnesylated GFP; another approach that we are testing is the CRErt2 model. IL-4+ MVs have showed the ability to promote the polarization of the microglia in vitro by the expression of a typical anti-inflammatory gene like YM1 and induce a slight reduction of the pro-inflammatory gene iNOS.

T12-12B

Influence of the anti-epileptic drug lacosamide (LCM) on glial properties in astrocyte/microglia co-cultures

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Background: Glial and neuronal expression of inflammatory mediators has been described in surgically resected brain tissue from patients with refractory epilepsy. Inflammation is accompanied by activation and proliferation of microglial cells. Astrocytes are coupled via gap junctions and have important function in neuronal trophic supply. Both, astrocytes and microglia are responsible for the cerebral immunological defense. However, the impact of anti-epileptic drugs (AED) on glial cells is relatively unknown and the influence of inflammatory parameters has not thoroughly been investigated. In this study, we examined the influence of the AED lacosamide (LCM) on the glial viability, the microglial phenotype, the astrocytic connexin 43 (Cx43) expression and functional coupling in a physiological and inflammatory modified *in-vitro* astrocyte/microglia co-culture model.

Methods: Primary astrocyte-microglia cultures were prepared from brains of postnatal (P0-P2) wistar rats and co-cultured with a physiological amount of 5% (M5) as well as 30% (M30) microglia in order to mimic inflammatory conditions. Co-cultures were treated for 24 hours with LCM at concentrations of 5, 15, 30 and 90 µg/ml. Viability was measured using the tetrazolium (MTT) assay. The microglial phenotype was determined by immunocytochemistry. The astrocytic Cx43 expression was measured by western blot and the functional coupling was detected by scrape loading technique.

Results: LAC did not influence the viability of the glial cells. However, LAC increased the amount of activated microglia phenotype and decreased Cx43 expression in M5 co-cultures in a dose-dependent manner. Furthermore, LAC decreased the amount of activated microglia phenotype and functional coupling and increased the Cx43 expression in M30 co-cultures.

Conclusion: Inflammation in the brain is accompanied by disturbance of glial cell functions. The results of this study demonstrate the dose-dependent influence of LAC on glial cells, who trigger inflammatory processes. We noticed significant microglial inactivation, after incubation of the M30 co-cultures. Therefore, we assume a beneficial therapeutic effect of anti-epileptic drugs (AED) with an anti-inflammatory glial potential in epileptic patients with persistent inflammation.

T12-13B

In mice retina contralateral to experimental glaucoma increased microglial cell number and retraction of microglial processes occurs beyond the GCL

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Question: To quantify retinal microglia signs of activation after two weeks of laser-induced ocular hypertension (OHT) in OHT-eyes and their contralateral eyes

Methods: Albino Swiss mice were divided into two groups, i.e. naïve (n=6) and lasered (N=9). Retinal whole-mounts were immunolabeled with anti Iba-1 to quantify microglia cell area and number.

Results: In naïve, contralateral and OHT-eyes microglia were distributed throughout the retina in the photorecector layer (PRL), outer plexiform layer (OPL), inner plexiform layer (IPL), nerve-fiber layer (NFL) and ganglion-cell layer (GCL). In comparison with naïve, OHT eyes and contralateral eyes had: i) a significantly higher cell number ($p<0.001$ $p<0.5$, respectively) and area of the retina occupied by microglial cells in the NFL-GCL ($p<0.001$ and $p<0.1$, respectively); and, ii) significantly less microglial arbor area in the OPL and IPL ($p<0.001$ in both instances for OHT-eyes) and ($p<0.05$ and $p<0.001$, respectively for contralateral eyes). The number of microglial cells was significantly higher in OHT eyes than in contralateral eyes ($p<0.001$).

Conclusions: Two weeks of laser-induced OHT provoked an increase in microglia number and retraction of microglial processes in OHT eyes and in contralateral eyes. In both instances such changes occurred beyond the GCL. Differences between OHT and contralateral normotensive untreated eyes could provide some insight into the pathophysiology of glaucoma.

T12-14B

Inflammasome expression in demyelinated CNS lesions

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Inflammasomes are multi-protein platforms in the cytosol that process pro-IL-1beta and pro-IL-18 into their active forms. Inflammasomes can be activated by endogenous danger signals, such as those released upon tissue damage.

Both IL-1beta and IL-18 are potent pro-inflammatory mediators that act on numerous cell types of the immune system as well as on the local tissue environment. In the CNS, astrocytes, microglia, neurons and oligodendrocytes express inflammasomes, as well as receptors for IL-1beta and IL-18, indicating the ability to respond to inflammasome activation.

Because inflammasomes are implicated in both tissue destruction and in the response to tissue damage, this study aims to specifically determine if inflammasome activation is a component of the local tissue response to myelin damage. To this end focal demyelinated lesions were induced in the spinal cord white matter of C57BL/6 mice and inflammasome components were analysed at different timepoints after the demyelinating insult.

The inflammasome component ASC was differentially expressed over time after myelin destruction, demonstrating that inflammasomes become activated following demyelination. Furthermore, IL-18 was differentially expressed at different timepoints after demyelination, demonstrating the bioactivity and functional significance of inflammasome activation in response to myelin damage.

As glial cells express receptors for IL-1beta and IL-18, and therefore can respond to inflammasome activity, we further analysed the direct effect of inflammasome derived IL-1beta and IL-18 on glial cells *in vitro*. While IL-18 had no effect on oligodendrocytes, IL-1beta promoted myelin protein production by

mature oligodendrocytes without altering the number of differentiated APC^+ oligodendrocytes. However, IL-1beta did not affect proliferation or survival of oligodendrocytes *in vitro*.

Our studies show that inflammasomes are expressed after a demyelinating insult in the CNS *in vivo,* demonstrating that inflammasomes are a key component of the CNS tissue response to myelin damage. *In vitro*, inflammasome-derived IL-1beta and IL-18 have differential effects on glial cells, and their individual roles after demyelination needs to be determined. Future studies will refine our understanding of inflammasome expression after demyelination and the effects of inflammasome products on different glial cells.

T12-15B

Temporal gene expression profile related to microglia reactivity in 3xTgAD mice

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Microglia are the cells responsible for an immune response within the central nervous system and have recently been associated with the progression of Alzheimer's Disease (AD). While some data point to a crucial microglia role in the inflammatory milieu of disease course, others showed that in aged brains these cells may lose their neuroprotective abilities rendering the brain to the emergence of neurodegenerative disorders. We recently found that primary cultures microglia become less responsive with age or in the presence of Amyloid-beta. Here, we decided to evaluate microglial response in an animal model of AD, the 3xTgAD.

Brain samples from wild-type and 3xTgAD animals were collected at 3, 6, 9 and 12-month and analyzed for mRNA expression of microglial response markers including phagocytosis-related protein, inflammatory proteins and inflamma-microRNA-155.

Microglia marker CD11b was markedly reduced at 3-months (0.5-fold) in 3TgAD animals, increasing afterwards at 6, 9 and with more pronounced values at 12-months (1.9-fold). Interestingly, the phagocytosis-related MFG-E8 was almost absent at 3-months 3xTgAD animals (0.1-fold), and kept reduced when compared to wild-type animals with disease progression suggesting a reduce microglia phagocytic ability. Concerning the inflammatory markers, only pro-inflammatory microRNA-155 was elevated at 3 and 6-months (4.0-fold), while other inflammatory markers such as TNF-alpha, IL-1beta and HMGB1 were down-regulated at these early stages (0.6-, 0.4- and 0.8-fold, respectively) but increased after 9-months, namely for HMGB1 (1.3-fold). Noteworthy, both typical M1 pro-inflammatory markers such as iNOS, MHC class II and SOCS1, or M2 anti-inflammatory/damage resolution markers including TGF-beta, Arginase1 and FIZZ1 were also reduced for the initial period of disease (3-months), augmented at 6 or 9-months but reduced once again at 12-months.

Our data suggests that in the 3xTgAD animal model microglia response is first refrained in the initial phase of disease (3-months), possibly to contain the damage, followed by an increased reactivity around 6 to 9-months, and decline once again at 12-months of animal life possibly indicating a more dystrophic/senescent microglia at this disease phase. Altogether, our results point to different microglia reactivity along AD course highlighting the need of distinct therapeutic strategies depending on the therapeutic window that may be addressed.

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T12-16B

Microglial Wnt signaling inhibition promotes microglia activation and oligodendrocyte maturation blockade

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Neuroinflammation is a key pathomechanism in numerous acute and neurodegenerative brain diseases, including encephalopathy of prematurity resulting from premature birth, which is the commonest cause of disability in children under 5 years. Microglia (MG) and macrophages (Mφ) are major players of neuroinflammation and in response to insult, MG/Mφ are capable of acquiring diverse phenotypes allowing them to participate in the cytotoxic response, immune regulation, and injury resolution.

To model the systemic inflammation observed in premature infants and that is associated with poor neurodevelopmental outcomes, we have developed a mouse model. We induce a chronic systemic inflammatory response by the neonatal intraperitoneal administration of IL-1β during a period corresponding to 28-34 weeks of human pregnancy (P1-P5). This model mirrors the main anomalies observed in premature infants: MG/Mφ activation, arrested maturation of oligodendrocytes leading to myelination defects, cognitive deficits, and MRI diffusion anomalies (Favrais et al., 2011).

The goal of this study was to characterize and unravel the molecular mechanisms controlling the MG/Mφ phenotypic profile in this model, allowing the potential identification of novel targets for neuroprotection.

In response to systemic IL-1β, brain MG/Mφ exhibited an immediate (4hrs) and sustained over time pro-inflammatory phenotype while a transient phenotype compatible with injury resolution was observed with a significant delay (>72hr). Transcriptome analysis (validated by qRT-PCR and Elisa) of MG/Mφ sorted from newborn brains revealed the down-regulation of multiple members of the Wnt pathway in activated cells. In primary cultures of MG, genetic or pharmacological inhibition of the Wnt pathway was sufficient to promote a pro-inflammatory phenotype while activation of the Wnt pathway prevented IL-1β-induced MG activation. In vivo MG/Mφ-specific deletion of β-catenin (the main effector of the canonical Wnt pathway) induced MG/Mφ activation, oligodendrocyte maturation arrest and myelin defect. Experiments currently underway in vivo will target MG/Mφ with nanoparticles (dendrimers) carrying a plasmid expressing a constituvely active form of β-catenin to ascertain if this can reduce the IL-1β-induced proinflammatory phenotype of brain MG/Mφ and associated myelin defects. Modulating Wnt signaling in brain MG/Mφ may be a promising therapy for neuroinflammation in preterm infants and, potentially, in other brain disorders involving MG/Mφ-mediated inflammation.

T12-17B

Astrocytes overexpressing transforming growth factor beta1 show increase uptake and degradation of beta amyloid

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Alzheimer's disease (AD) is the most common type of dementia and accounts for an estimated of 60% to 80% of the cases. TGFbeta1 was found to be increased in Alzheimer's patients CSF and serum. Furthermore, there is a co-localization of TGFbeta1 with beta amyloid plaques (Abeta) in the brains of AD patients. Interestingly, cross bred of mice that over express TGFbeta1 under the promoter of astrocytes together with APP mice that over express Abeta plaques in the brain showed a reduction of parenchymal plaques. Astrocytes are essential for the maintenance of the brain homeostasis. Here we aim to investigate TGFbeta1 signaling in astrocytes in order to define its role in their activation in AD. We found that adult astrocytes isolated from overexpressing TGFbeta1 mice are more activated and exhibit higher levels of soluble $Abeta_{1-42}$ uptake compared to astrocytes isolated from WT mice. Furthermore, we discovered that astrocytes over-expressing TGFbeta1 significantly degrade more Abeta plaques versus WT astrocytes. In addition, we discovered that TGFbeta1 has also paracrine effect on astrocytes towards Abeta uptake. Understanding astrocytes' specific TGFbeta1 signaling in Alzheimer's disease may open a new avenue for therapeutic intervention.

T12-18B

A statistical physics-based spatial analysis in APP/PS1 mice reveals that astrocytes do not migrate to amyloid-beta plaques

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Objective: The clustering of GFAP immunopositive astrocytes around amyloid-beta plaques in Alzheimer's disease has led to the widespread assumption that plaques attract astrocytes. However, recent studies show that astrocytes stay put in injury. Here we re-examine whether astrocytes migrate to plaques by mathematically analyzing astrocyte topology in APP/PS1 mice and wild type littermates.

Methods: Our approach has three improvements over GFAP *postmortem* immunohistochemistry. First, we used 3D reconstructions of images captured *in vivo* through cranial windows by 2-photon microscopy. These materials are superior to sectioned specimens from fixed brains because they preserve true spatial relationships in 3D to great depths (up to 200 microns from the cortical surface), which allowed us to obtain accurate positional information for each astrocyte. Second, astrocytes were labeled with sulforhodamine 101, a selective fluorescent marker of reactive and non-reactive astrocytes, thus avoiding the bias of identifying only a subset of astrocytes as with GFAP. Third, astrocyte and plaque interactions were examined by two mathematical functions: the pair-correlation *g(r),* and the characteristic length (L_C) of Voronoi cells, which combine global and plaque-centered perspectives, and allow for quantitative comparisons to be made. Finally, simulations were used to help interpret astrocyte-to-astrocyte and astrocyte-to-plaque contact interactions, and to investigate the effect on astrocyte topology of plaque loads in the upper limit of what could be present in aged humans.

Results: We found that, in wild type mice, cortical astrocyte topology fits a model akin to a liquid of hard spheres that exclude each other in a confined space. Plaques do not disturb this arrangement except at very large plaque loads but, locally, they cause subtle outward shifts of the astrocytes located in three tiers around plaques.

Conclusion: Astrocytes respond to plaque-induced neuropil injury mostly by changing phenotype, and hence function, rather than location.

T12-19B

Role of Tumor Necrosis Factor Receptor 2 signaling in microglia and macrophages in experimental autoimmune encephalomyelitis

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Tumor necrosis factor (TNF) is a pleiotropic cytokine involved in numerous physiological and pathological processes, including multiple sclerosis (MS). It exists in two forms, transmembrane (tmTNF) and soluble (solTNF). The biological functions of TNF are mediated by TNFR1 and TNFR2: solTNF signals preferentially via TNFR1 promoting inflammation and apoptosis, and tmTNF via both TNFR1 and TNFR2 promoting anti-inflammatory and remylinating effects. To date, most studies have focused on TNFR1, and the role of TNFR2 in health and disease is poorly understood. Since TNFR2 is expressed in microglia and macrophages, and both participate in the pathophysiology of MS, we sought to elucidate the functions of TNFR2 in these cells in vivo by generating novel LysM-cre:TNFR2$^{fl/fl}$ mice with conditional ablation of TNFR2 from both populations. In naïve conditions LysM-cre:TNFR2$^{fl/fl}$ mice did not display phenotypical abnormalities. After induction of EAE, LysM-cre:TNFR2$^{fl/fl}$ mice developed significantly delayed and suppressed disease, associated with reduced numbers and activation of splenic lymphocytes, reduced CD4 T cell infiltration into the spinal cord, improved myelin sparing and axon preservation. Interestingly, we observed that, despite the lower

disease severity, infiltrated B cells in the spinal cord of LysM-cre:TNFR2$^{fl/fl}$ mice expressed higher levels of MHC-II compared to controls, and infiltrated CD4 and CD8 T cells showed enhanced capacity to secrete pro-inflammatory IL17, TNF and IFNg. To gain insight into the role of TNFR2 in microglia versus macrophages, we generated bone-marrow chimeric mice with TNFR2 ablation in either population: LysM-cre:TNFR2$^{fl/fl}$-->WT chimeras (macrophage-specific), and WT LysM-cre:TNFR2$^{fl/fl}$-->chimeras (microglia-specific). Macrophage-specific chimeras showed suppressed EAE similarly to LysM-cre:TNFR2$^{fl/fl}$ mice, while microglial-specific chimeras showed significantly exacerbated EAE. This latter result was confirmed in CX3CR1-cre^{ER}:TNFR2$^{fl/fl}$ mice, with inducible conditional TNFR2 ablation exclusively in microglia.

Though microglia and macrophages are closely related in their physiological and pathological functions, here we demonstrate that TNFR2 activation in these populations plays opposite roles in EAE etiopathology: detrimental in macrophages, protective in microglia. Our data underscore that TNFR2 functions are cell-dependent and cell-specific conditional knockout models represent an invaluable tool to better understand TNFR2 signaling and function in health and disease.

T12-20B

Synaptophysin is a suitable marker to study axonal transport damage during experimentally induced demyelination

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Synaptophysin is an abundant protein in membranes of synaptic vesicles. It is an accepted marker to study synaptic activity and was previously shown to be down-regulated in demyelinated CNS lesions in multiple sclerosis (MS) patients. Here, we have used the toxic cuprizone model of demyelination and the inflammatory model Theiler virus encephalomyelitis (TMEV) to study the role of synaptophysin in demyelination.

In our study we could not observe any alterations in mRNA expression levels of synaptophysin and the post synaptic protein PSD-95 in cortical neurons during cuprizone induced demyelination. We found co-localization of synaptophysin with the marker APP which is used to detect axonal damage. The appearance of synaptophysin positive spheroids correlated with numbers of activated microglia in the corpus callosum and was linked to the up-regulated expression of pro-inflammatory cytokines (TNF-α and Il-1ß). The size of synaptophysin bulbs varied during the course of demyelination. Small (1-2µm) and middle sized (2.5-7.5µm) spheroids predominated in the inflamed corpus callosum during the peak of microglial accumulation and myelin loss. Large (8-15µm) synaptophysin bulbs were also found and persisted during the complete course of demyelination and were even found up to 2 weeks after demyelination was stopped and remyelination occurred. We suggest that small vesicle jams could be removed but that the large vesicles remained in transected axons. The results were confirmed in Theiler virus encephalomyelitis where we could detect synaptophysin/APP positive spheroids in demyelinated areas of the spinal cord. In order to investigate if a dysregulation of axonal motor proteins could be responsible for the accumulation of synaptophysin we studied the expression of these proteins by qPCR. Interestingly, mRNA expression of the anterograde motor proteins such as KIF 1a, 1b, 5a, and 5b and the retrograde motor protein dynein were not regulated in cortical neurons during de- and remyelination.

In conclusion synaptophysin is a suitable marker which might be used to study axonal transport disturbances/damage during experimentally induced demyelination.

T12-21B

CNS endothelial IL-1 signaling drives neuroinflammation

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The pro-inflammatory cytokine interleukin (IL)-1 is crucially important for mediating the infiltration of inflammatory cells in multiple sclerosis (MS) and its animal model, experimental autoimmune encephalomyelitis (EAE). IL-1 signaling in endothelial cells (ECs) and glial cells of the blood-brain barrier (BBB) induces an up-regulation of pro-inflammatory molecules, which in turn leads to the recruitment of leukocytes to the CNS. Changes in expression of the IL-1 receptor and/or receptor antagonist have been implicated in MS, as high levels of IL-1 have been found in the brain white matter of these patients. Moreover, in mice globally lacking either IL-1 or the signal transducing receptor (IL-1R1), as well as in wild type mice treated with IL-1 inhibitors, a delayed onset of EAE with overall reduced disease severity was observed. In this project we are interested to investigate the importance of IL-1 signaling at the level of the BBB, specifically in ECs and astrocytes, and its consequences for EAE autoimmunity in the CNS. In unchallenged wild type mice we could detect 5% of CNS ECs staining positive for IL-1R1 as assessed by flow cytometry. In contrast, already at three days after EAE induction we found a significant up-regulation of the receptor with 20-25% positive cells. This level remained stable until day 15 post immunization. To study the EC-specific role of IL-1 signaling we make use of the conditional gene targeting approach to create a novel mouse strain, where the IL-1R1 is specifically deleted in ECs of the BBB, using the tamoxifen-inducible SLCo1c1-CreER^{T2} recombinase (IL-1R1^{SLC} mice). When we immunized these mice we did observe a significantly reduced disease course with similar time of onset but significantly lower peak disease score, compared to control mice. Although the overall proportions of CNS-infiltrating cells at the peak of disease were very similar between the two groups, we did find differences in cytokine expression levels of infiltrating T cells. MOG antigen-specific T cells in the brain of IL-1R1^{SLC} mice produced less GM-CSF than cells in the brain of control mice. Similarly, in the corresponding spinal cords we found less GM-CSF- as well as interferon γ-producing T cells in experimental versus control mice, suggesting that IL-1 signaling in CNS ECs orchestrates the infiltration of pathogenic T cells during EAE. At this point we conclude that IL-1 signaling in ECs of the BBB significantly contributes to the induction of EAE, the underlying molecular mechanisms of which are investigated in the frame of this project.

T12-22B

Intrahippocampal clodronate administration alters the brain inflammatory response to systemic LPS in mice

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Microglia, in case of environmental challenge, is known to have neurotoxic effects some of which have been associated with chronic brain disorders such as depression. One of the common animal models of infection is peripheral administration of lipopolysaccharide (LPS), which induces depression-like behaviour in rodents through the release of pro-inflammatory cytokines in the brain. Some studies have associated these inflammatory changes in the brain to activation of microglial cells. In the current study we investigated the specific contribution of these cells to the overall CNS inflammatory response in mice treated with an injection of LPS. For this, we depleted phagocytic microglia using liposome-mediated intracellular delivery of dichloromethylene-bisphosphonate (Clodronate) injected in the CA3 region of the hippocampus prior to LPS. Hippocampal CA3 region is associated with changes in cognitive behaviour in depression as well as susceptibility to changes in inflammatory milieu. As expected, LPS induced a significant increase in microglia activation abolished by the Clodronate treatment in the CA3 region. Inflammatory mediators expression analysis revealed a significant increase in the pro-inflammatory IL-1ß as well as in the chemokines Kc1 and Cxcl2 all of which were potentiated by the Clodronate treatment. Conversely, Clodronate completely abolished the increase in IL-10, an anti-inflammatory cytokine. In addition, LPS induced an increase in the expression of the tryptophan degrading enzyme indoleamine-2,3-dioxygenase (IDO) used as a correlative measure of depression-like behaviour. These data suggest that microglia may be important effectors underlying the inflammatory changes associated with chronic behavioural alterations such as those associated with depression in humans.

T12-23B

The role of mTOR kinase in glioma-activated rat microglia and in human glioma

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Question: Increased activation of mammalian target of rapamycin (mTOR) is observed in numerous human cancers. Recent studies on the glioma kinome have identified several deregulated pathways that converge and activate mTOR. In parallel the microglial polarization and its dual role, tumoricidal versus a role favoring tumor growth is coming central in the study of human brain cancers. Recently we investigated the effects of different environment on microglia polarization. **Methods**: In the present study, we have compared the effects of μM concentrations of rapamycin (RAPA) and its analog, RAD001 (RAD), on activated rat microglia; the latter was obtained by exposing cells to conditioned medium harvested either from inflammatory activated glioma cells (LI-CM) or from glioma cells kept under basal conditions (C-CM). In addition we are currently underway on 37 surgical specimens of glioblastoma multiforme to evaluate in the center and in the periphery of glioma the microglial polarization and mTOR activation. **Results**: We show that the inhibition of mTOR polarizes glioma-activated microglial cells towards the M1 phenotype and in parallel reduces the induction of a M2 status. In fact RAPA and RAD significantly increased iNOS expression and activity, while on the same time significantly reducing IL-10 gene expression induced by C-CM. Similar results were obtained using the conditioned media obtained after glioma stimulation with LPS-IFNγ (LI-CM), which was found to induce a mixture of M1 and M2a/b polarization phenotypes. In these conditions, the inhibition of mTOR led to a significant up-regulation of iNOS, and in parallel to the down-regulation of both ARG and IL-10 gene expression. In brain human glioma we note an high percentage of microglia/macrophage cells with different polarization, but all the studied cases are positive for mTOR antibody. **Conclusions**: These data suggest that mTOR inhibition could have a central role to drive the polarization of microglial cells in glioma versus a M1 status respect to a M2 phenotype.

T12-24B

Oligodendroglial TNFR2 mediates transmembrane TNF-dependent repair in experimental autoimmune encephalomyelitis by promoting oligodendrocyte differentiation

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Tumor necrosis factor (TNF) has been associated with the pathophysiology of multiple sclerosis (MS). TNF exists in two forms, transmembrane (tmTNF) and soluble (solTNF), whose functions are mediated by TNFR1 and TNFR2. SolTNF primarily signals through TNFR1, and tmTNF through both TNFR1 and TNFR2. The cellular processes activated by the two receptors are often opposite: TNFR1 mediates apoptosis and inflammation, whereas TNFR2 is associated with cell survival, immunity and myelination. Numerous studies have linked MS to the detrimental effects of solTNF and TNFR1, while tmTNF is protective and important for repair and remyelination.

Here we demonstrate that TNFR2 expressed in the oligodendrocyte lineage is a key mediator of the protective functions of tmTNF in EAE. CNP-cre:TNFR2$^{fl/fl}$ mice with conditional ablation of TNFR2 in oligodendrocytes showed exacerbation of EAE, with increased axonal damage and myelin pathology, as well as reduced remyelination. The EAE clinical profile was not improved by treatment with the solTNF inhibitor XPro1595, which suppresses EAE in TNFR2$^{fl/fl}$ control mice, indicating that for tmTNF to exert its beneficial effects a functional TNFR2 in oligodendrocytes is required. Furthermore, in vitro studies with primary oligodendrocyte cultures from WT and TNFR2$^{-/-}$ mice demonstrated impaired differentiation from precursors into pre-myelinating oligodendrocytes in the absence of TNFR2, suggesting that TNFR2 is directly modulating pathways necessary for oligodendrocyte differentiation. To better address this point, we evaluated the expression of microRNAs in naïve conditions and after

EAE and found differential expression of various microRNAs, many of which are known regulators of oligodendrocyte proliferation and differentiation, as well as inflammation. More specifically, we found that members of the miR-219, miR-138 and miR-338 clusters were downregulated following EAE in CNP-cre:TNFR2$^{fl/fl}$ mice but not TNFR2$^{fl/fl}$ controls, indicating a failure in activating crucial signals for oligodendrocyte differentiation and remyelination in disease conditions. Taken together our data provide the first direct in vivo evidence that TNFR2 signaling in oligodendrocytes is important in driving oligodendrocyte differentiation, thereby sustaining the tmTNF-dependent repair process in neuro-immune disease. Our studies identify TNFR2 in the CNS as a viable molecular target for the development of remyelinating agents for progressive MS, addressing the most pressing need in MS therapy.

T12-25B

Liver X receptor activation in MS lesions

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Multiple sclerosis (MS) is an autoimmune disease of the central nervous system (CNS). It is pathologically characterized by infiltrating macrophages that destroy the myelin sheaths. Myelin consists for a large part of cholesterol and myelin breakdown causes an overload of cholesterol and oxysterols in macrophages. These oxidized derivatives of cholesterol are able to activate liver X receptors (LXRs), which will lead to the subsequent induction of genes involved in cholesterol efflux, including *ABCA1, ABCG1* and *APOE*. Previously, we demonstrated that myelin activates LXRs in primary murine macrophages. In this study we determined the expression of LXRs and their response genes in human phagocytes after myelin phagocytosis and in MS lesions. Myelin ingestion induced the LXR response genes *ABCA1* and *ABCG1* in human monocyte derived macrophages, demonstrating myelin activates LXRs in human phagocytes. Next, we used real-time quantitative PCR (qPCR) and immunohistochemistry (IHC) to determine mRNA and protein levels of LXRs and their response genes. We found that both *ABCA1* and *APOE* gene expression and protein levels are highly upregulated in active MS lesions compared to healthy controls. MHCII-positive infiltrating macrophages and microglia in active lesions are positive for ABCA1 and APOE, indicating LXRs are activated in these myelin phagocytosing cells. LXRα is mainly present on MHCII+ cells while LXRβ is predominantly observed in perilesional astrocytes. Our findings indicate that LXRs are activated in phagocytes and perilesional astrocytes in active MS lesions. Future studies are needed to determine the impact of LXR activation on infiltrating and residential CNS celtypes.

T12-26B

Multiple Sclerosis: studying lipocalin 2 as a novel player in the pathophysiology of the disease

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Multiple sclerosis (MS) is a chronic progressive inflammatory disease of the central nervous system (CNS). In developed countries, it is the second cause of neurological disability in young adults, with high burden for the patient, the family and the resources of the health system. Multiple sclerosis is a complex disease in which there is a strong immune response against the myelin sheath of CNS axons, but its underlying mechanisms are only partially understood. One of most notable aspects of the disease is the migration of peripheral inflammatory cells through the brain barriers towards the CNS. Recently, we published strong evidence in human MS patients and in animal models of MS, that the protein lipocalin 2 (LCN2) is involved in disease pathophysiology. In the experimental autoimmune encephelomyelitis (EAE) mouse model of MS we observed increased expression of the gene encoding for LCN2 in the choroid plexus (CP) - cerebrospinal fluid (CSF) brain barrier. We also demonstrated that the levels of this protein in the CSF increased in the active phases of the disease, an increase that was abrogated by treatment with natalizumab (a drug used for treating the disease in humans, that

blocks leukocyte entry into the CNS). Interestingly, immunostaining revealed that neutrophils infiltrating the CP were the source of the increased *Lcn2* expression in this structure; yet, LCN2 was also detected in astrocytes, solely in the active phases of the disease, particularly in the brain regions typically affected in MS patients. In agreement with data from mice, we observed that MS patients present increased CSF LCN2 levels. Altogether, these findings support LCN2 as a valuable molecule for the diagnostic/monitoring of MS and suggest its potential involvement as a disease modulator. In accordance, recent studies by others showed that EAE is more severe in LCN2-null mice, which is suggestive of a protective role for LCN2 in the progression of the disease. Interestingly, in the context of the present application, it is relevant to highlight that LCN2 has also been described to promote apoptosis and proliferation, depending on its role in removing or delivering iron to cells, respectively; and to stimulate the synthesis of brain chemokines. It is possible that through these pathways LCN2 may participate in key process of the MS disease such as: myelin formation (though iron levels modulation and proliferation of brain cells, specially oligodendrocytes) and immune cell migration into the brain. Altogether, these data lead us to hypothesize LCN2 as a modulator of the MS clinical phenotype.

T12-27B

The effect of microglia on progenitor cells during tuberal hypothalamic development

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Microglia influence during central nervous system (CNS) inflammation and injury is an active area of research. However, new research has shown microglia begin to invade the embryo brain around embryonic (e) day 10.5 in mouse, suggesting a unique role for these yolk-sac derived immune cells in CNS development. Although the role of microglia in brain development in utero is just starting to be explored, early reports suggest microglia can influence neurodevelopmental processes such as progenitor maintenance and cell differentiation. Our overall research is focused on asking three questions: (1) what are the microglia populations present during development; (2) what mechanisms direct and attract microglia towards the developing fetal brain; (3) what functional role does microglia play in the developing hypothalamus.

I have systematically identified the timing of microglia invasion into the developing hypothalamus and microglia activation state using fluorescent imaging approaches. The developing hypothalamus is an excellent system to study microglia invasion and microglia-cell interactions due to its easily identifiable progenitor zone and readily available markers that label distinct nuclei and neuronal populations. We have defined the spatiotemporal timing of microglia invasion in the developing embryonic tuberal hypothalamus and are now determining the influence of microglia on developing cell populations by using pharmacological microglia knock down models. Specifically, pregnant CD1 mice were sacrificed and embryonic brain tissue was harvested on e11.5, e13.5, e15.5 and e17.5. Cryosectioned brains were labeled with microglia and hypothalamic markers for fluorescent imaging followed by stereological analyses employed to quantify the hypothalamic microglia population.

We show microglia invade the hypothalamus starting at e11.5 and align with hypothalamic progenitors near the end of neurogenesis. We are currently exploring on our pharmacological knock down model to determine the function of microglia during hypothalamic development.

Combined, these data suggest a possible role for microglia in maintaining hypothalamic progenitor populations given their pivotal position during development, where they can interact with progenitors. Defining the role of microglia during normal fetal development will allow for a better understanding of how *in utero* perturbations can affect microglia function during key windows in hypothalamic embryonic development.

T12-28B

A novel imaging approach to monitor multiple sclerosis

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Introduction: The neuroinflammatory reaction led by microglia/macrophages is closely associated with axonal loss as well as with the inhibition of the regeneration and recovery of oligodendrocytes in multiple sclerosis (MS) [1]. Likewise, the release of glutamate by microglia/macrophage through the cystine/glutamate antiporter (xc^- system) could be involved in the pathophysiology of MS (Domercq et al., 2007; Pampliega et al., 2011). *In vivo* imaging of xc^- system has been scarcely explored to date despite imaging modalities have provided valuable information about inflammation underlying MS.

Methods: I*n vivo* positron emission tomography (PET) studies with [^{18}F]FDG, [^{11}C]PK11195 and [^{18}F]FSPG were performed to explore changes in metabolic activity, TSPO (activated microglia) and xc^- system activity before (day 0) and at 7, 14, 21 and 28 days after experimental autoimmune encephalomyelitis (EAE) in rats. Likewise, T1 weighted magnetic resonance imaging (MRI) and spectroscopy brain studies were carried out in EAE rats to assess ventricle volume and the spectrum of metabolites.

Results: Immunized rats developed typical clinical signs of acute EAE, starting over day 10 and followed by disease progression that peaked at day 14. EAE symptoms persisted for seven days and remitted from day 21 to day 28 after EAE. [^{18}F]FDG PET and MRI spectroscopy did not show significant changes in both metabolic activity and metabolites in central nervous system (CNS) after EAE. Despite these findings, a significant increase of activated microglia/macrophages with [^{11}C]PK11195 was observed in cerebellum and spinal cord that stand in agreement with the clinical outcome profile presented by EAE rats. Likewise, ventricular volume experienced a significant increase at day 14 followed by a decrease later on. In addition, [^{18}F]FSPG PET showed a significant binding increase two weeks after immunization in the lumbar spinal cord, the region more severely affected in this model, evidencing the capability of [^{18}F]FSPG to discriminate spinal cord regions according to the damage. Finally, treatment with chlodronate reduced microglial cells, as revealed by immunohistochemistry, and [^{18}F]FSPG binding, demonstrating the role of system xc- on the inflammatory reaction.

Conclusions: Taken together, these results suggest that [^{18}F]FSPG might be a suitable novel radiotracer for monitoring MS progression and therapeutic response.

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T12-29B

Elucidating the roles of FGF signaling in Multiple Sclerosis

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Fibroblast growth factor 9 (FGF9) plays important roles in coordinating the proliferation, differentiation and survival of neurons and glia in the developing nervous system, but it is only now becoming apparent this pleiotropic growth factor may also play an important role the pathogenesis of multiple sclerosis(MS). Our data demonstrate FGF9 is up regulated in active MS lesions where it appears to represent a predominately oligodendroglial response to inflammatory demyelination. *In vitro* studies demonstrate FGF9 is a powerful inhibitor of (re)myelination, but this is not mediated by a direct effect of FGF9 on cells of the oligodendrocyte lineage, but in response to soluble products secreted by FGF9

signal transduction in astrocytes. Transcriptional profiling of the effects of FGF9 in myelinating cultures demonstrates it induces complex downstream changes in gene expression that result in a pro-inflammatory signalling environment in which (re)myelination fails. These effects are associated with marked increases in expression *Spry2, Spry4, Dusp5,* and *Dusp6*, four well characterised negative feedback inhibitors of FGF signalling. In order investigate the pathophysiological significance of these *in vitro* observations we are exploring expression of selected candidates in MS lesions. This confirmed FGF9 expression is up regulated in oligodendrocytes, and to a lesser extent in astrocytes, in both active acute lesions and at the active rims of chronic active lesions and that this is associated with increased expression of Sprouty2 and Sprouty4 by astrocytes. In contrast Sprouty2/4 expression was minimal in normal appearing white matter adjacent to these lesions and in white matter from healthy controls. These findings provide further evidence that FGF signalling in astrocytes plays a role in the development of MS lesions. Elucidating the function of *Spry2/4* during lesion development will help determine how the astrocytic response in MS may be manipulated to promote remyelination; a strategy predicted to restore function and prevent further axonal loss in MS.

T12-30B

Probenecid application prevents clinical symptoms and T cell infiltration in a mouse model of experimental autoimmune encephalomyelitis

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Multiple sclerosis (MS) is a chronic inflammatory disease of the central nervous system, affecting primarily oligodendrocytes. Demyelination and neuroinflammation lead to axonal damage and subsequently to neurological impairments. Immunological contributors are T cells, particularly CD4+ cells, which differentiate amongst others into proinflammatory type 1 T helper (Th1) cells and enter the central nervous system via MS-associated blood-brain-barrier lesions [1]. Recently, CD8+ cytotoxic T cells have also been implicated in the pathogenesis of MS. T cell activation itself is mediated by the release of adenosine triphosphate (ATP), and this process involves purinergic receptors as well as pannexin (Panx) proteins [2].

As Panx1 is expressed on both CD4+ and CD8+ T cell subsets [3], we here propose that inhibition of pannexins will prevent T cell activation and subsequently MS symptoms. We therefore investigated whether application of probenecid, a known pannexin inhibitor, will prevent the onset of clinical symptoms in a mouse model of MS, the experimental autoimmune encephalomyelitis (EAE) model.

EAE was induced in female mice by application of a myelin oligodendrocyte glycoprotein peptide (MOG_{35-55}). EAE-induced mice received either no treatment or daily intraperitoneal injections of probenecid (100 mg/kg body weight) or solvent from day one onwards. Ascending paralysis, the main clinical symptom in EAE, was evaluated using a scoring system. Clinical scores, histological scores, total T cell numbers (determined by CD3) and microglia activation (determined by CD68 expression) were compared between experimental groups.

EAE-induced mice (no treatment) displayed increasing clinical scores from day 8 onwards. Solvent treatment did not change any of the parameters investigated. In contrast, probenecid treatment resulted in significantly lower clinical scores, which were indistinguishable from those of control and sham animals (no EAE induction). Probenecid-treated animals also displayed significantly fewer T cells within the spinal cord. Microglia activation, however, was similar between groups. Taken together, systemic application of probenecid prevented the onset of EAE, associated with a reduction in inflammatory lesions.

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T12-31B

Microglia - the radio-resistant immune cell of the brain

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Microglia, regarded as the brain´s immune cells, migrate from the yolk sac to the brain during embryogenesis and remain during adulthood. Whereas other mononuclear populations are substituted or replaced by migrating cells from the blood or the bone-marrow, microglia mainly reside because of their long time survival and the high capability of self-renewal. Another characteristic of microglia is their high radio-resistance, already shown in vivo. Here, we tested whether microglia maintain radio-resistant even in vitro as single cell, implying intrinsic anti-apoptotic or repair-related mechanisms independent of their local environment.

Therefore we irradiated brain ($CD45^{high}$ and $CD45^{int}$) and spleen mononuclear cells ($CD45^{high)}$ straight after isolation with 30 Gy of X-ray and, after 4h, sorted these cells for the leucocyte marker CD45, the cell death marker PI and the marker for early apoptosis Annexin V. Furthermore, we investigated the mRNA-expression pattern of several apoptosis and repair related genes.

The FACS analysis revealed that splenocytes and brain macrophages ($CD45^{high)}$ are highly sensitive to 30 Gy of X-ray, whereas microglia ($CD45^{int}$) are resistant. Furthermore, we found several genes that were significantly and contrasting regulated in microglia compared to brain macrophages and splenocytes.

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T12-32B

Glial changes in psychiatric disorders; towards isolating glia from the post-mortem human brain

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Roughly one third of the European population is afflicted with one or more psychiatric disorders, which in most cases goes untreated. Presenting an enormous social and economic burden, research into the pathological basis, and therefore possible therapeutic strategies, have increased dramatically in recent years. In order to provide researchers worldwide with well-characterized post-mortem brain material from different psychiatric disorders, the Netherlands Brain Bank (NBB) recently introduced the NBB-Psy project (www.nbb-psy.nl). Psychiatric patients and healthy controls are prospectively registered as post-mortem brain donors, resulting in a very short post-mortem delay. Besides providing frozen and fixed brain tissue for immunohistochemistry, we are currently investigating the possibility to acutely isolate primary glial cells (microglia and astroglia) from post-mortem brains and to make these available for researches through the NBB facility. For microglia, a rapid isolation protocol with high yield, through use of CD11b capturing beads, enabling phenotyping with FACS was already developed previously.[1] We are currently investigating whether primary microglia can be frozen and stored and if functional in vitro experiments can be performed after thawing. Due to the lack of known cell surface markers for human astrocytes, the acute isolation of astroglia remains challenging. Other possibilities to create astrocyte-enriched primary cultures are the use of fluorescent probe-uptake or specific astrocyte favoring culture conditions. When both glial types can be reliably isolated from post-mortem brains, we will start to immortalize glial cells isolated from both controls and patients, in order to facilitate more high-throughput analysis of glial cell function.

Through a collaboration with Erasmus University Rotterdam, iPSC-derived glial cells will furthermore be generated from patient and control-derived skin fibroblasts, to further complement the growing number of scientific tools to study glial changes in psychiatric disorders. Combined with a growing list

of brain donors, NBB-Psy aims to quickly gain an increased understanding of the pathological substrate of psychiatric disorders, and much needed new insight into therapeutic targets.

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T12-33B

Inflammatory response caused by GFAP mutations in Alexander disease

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Alexander disease (AxD) is a primary disorder of astrocytes, caused by heterozygous mutations in *GFAP*, which encodes the major astrocyte intermediate filament protein, GFAP. Astrocytes in AxD accumulate massive amounts of GFAP and undergo many changes, including the inhibition of proteasomal activity, activation of MAP kinase stress pathways, the up-regulation of small heat shock protein genes, the loss of the ability of astrocytes to buffer glutamate and potassium and the activation of mTOR kinase. AxD has historically been termed a "leukodystrophy" due to the so characteristic myelin degeneration and has never been categorized as an inflammatory disease. Here we show that AxD has a very strong inflammatory component principally involving astrocytes and microglia. We have used the $GFAP^{Tg.}$;$Gfap^{+/R236H}$ mice of AxD that recapitulates closely many of the human pathological features. The life span of these mice is of about 28-33 days and they die with convulsive seizures. They show weakness, reduced body weight and struggle to use the front limbs. They accumulate Rosenthal fibers (large aggregates of GFAP and small heat shock proteins characteristic of human AxD) in several brain regions. We have focused on the hippocampus and spinal cord since they display marked pathology and are associated with seizures and motor problems respectively. We found that hippocampus and spinal cords of $GFAP^{Tg}$;$Gfap^{+/R236H}$ mice show a robust increase in microglia and levels of Iba1 in both regions at 2 and 4 weeks of age. We also found major increases in CCL2, CXCL10, soluble ICAM, and CXCL1 among others by non-quantitative ELISAs. A modest increase in T lymphocytes was also found especially around blood-vessels and associated with meninges in both regions. We have observed similar T cell infiltration in AxD brains. We believe that one of the consequences of the astrocyte cell stress reaction is the production of inflammatory molecules and the further activation of microglia.

T12-34B

CD163+ Macrophages in human ischemic stroke

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Objective: CD163 is a scavenger receptor expressed in brain perivascular and meningeal macrophages that have been attributed a function in immune surveillance. Expression of CD163 has also been described in alternatively activated M2 macrophages. A soluble form (sCD163) has been reported in the blood. We aimed to examine the response of this particular subset of macrophages in human ischemic stroke.

Material and Methods: Blood samples of ischemic stroke patients were obtained at admission and on days 1, 2, and 90. CD163 was studied in monocytes by flow cytometry in 95 patients and serum sCD163 was studied by ELISA in 16 patients and 12 control subjects. The brains of five acute ischemic stroke patients who died between 1-15 days after stroke onset at the Stroke Unit of the Hospital Clinic of Barcelona were also used in this study. Written consent was obtained from their

families for tissue removal after death for diagnostic and research purposes at the Neurological Tissue Bank of the Biobank-Hospital Clinic-IDIBAPS. Immunofluorescence was carried out in cryostat tissue sections and the number of CD163+ cells/area was counted. The brain tissue of one patient was studied by flow cytometry. The study was approved by the Ethics Committee of this Hospital.

Results: CD163 was mainly expressed in $CD14^{hi}$CD16+ monocytes ($p<0.001$) that increased in number at day 7 ($p<0.05$). The serum of stroke patients showed higher sCD163 at admission (4.1 mg/L) compared to day 90 (2.9 mg/L) and controls (2.6 mg/L) ($p<0.05$). In the necropsies, we detected a time-dependent increase in the number of CD163+ cells in the meninges proximal to the ischemic core but not in distant meninges ($p<0.05$). In one patient (deceased at day 5), flow cytometry showed more CD14+CD163+ cells in the infarct periphery and ipsilateral periventricular area than in the core, and in the meninges proximal than distal to the core. CD163+ cells were not detected in the brain parenchyma from 1 to 5 days. However, at day 15, CD68+CD163+ macrophages were abundant in the ischemic core but not the periphery.

Conclusion: The subtype of $CD14^{hi}$CD16+ blood monocytes expressed CD163. Stroke increased the number of CD163+ monocytes and the amount of sCD163, which seems to act as an acute phase reactant. In the brain tissue, the results suggest that CD163+ macrophages invade the ischemic core several days after stroke and the meninges and/or ventricles are gateways for access of CD163+ cells to the ischemic tissue

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T12-35B

Sonic hedgehog and vitamin D modulation of metalloproteinase expression and in vitro endothelial junction integrity

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Multiple Sclerosis (MS) is an inflammatory autoimmune disorder caused by the presence of multifocal demyelinating lesions within the CNS. Blood brain barrier (BBB) disruption, in part driven by cytokines, is an early, crucial event in the pathogenesis of MS, as it facilitates the infiltration of activated immune cells into the CNS. Astrocyte-derived sonic hedgehog (Shh) promotes BBB formation and a reduction in Shh levels has been reported in MS patients. Inhibition of the Shh pathway can induce BBB breakdown by disruption of tight junctions in endothelial cells forming the BBB in vivo. Low circulating levels of vitamin D3 have been associated with an increased risk of MS and dietary vitamin D3 supplementation in MS treatment is increasingly adopted, although the molecular pathways underlying vitamin D3 effects on MS pathogenesis have not been fully clarified. Previously vitamin D3 has been shown to reverse symptoms in experimental autoimmune encephalitis, involving a calcium-dependent mechanism and also to modulate metalloproteinase expression in several cell types. Interplay between Shh and vitamin D3 pathways has been reported in other cell types but this has not been yet studied in the context of the BBB.

We hypothesised that vitamin D3 may modulate metalloproteinase gene expression in endothelial cells, during Shh-driven BBB formation in an in vitro model system. Vitamin D3 can modulate tight junctional integrity in epithelial cells and here we have explored its role in microvascular endothelial cell-cell junction maintenance in vitro. We observed that cytokines induced the expression of MMP9 and the presence of cytokines and cyclopamine, a disrupter of hedgehog signalling, resulted in further regulation of MMPs -2 and -14 and ADAM17/TACE. These changes in gene expression were prevented by co-stimulation with Shh. Vitamin D3 also increased expression of ADAM17 and TIMPs 1 and 2, but suppressed that of MMP13. MMP13 has previously been shown to disrupt endothelial barriers possibly through fragmentation of Z0-1.Vitamin D3 in combination with Shh had a protective role in maintenance of Z0-1 expression (assessed by quantitative immunofluorescence at tight junctions) following stimulation of endothelial monolayers with cytokines. Current experiments are aimed at determining the role of vitamin D3 and Shh in endothelial cell permeability in an in vitro BBB model system.

In conclusion the interplay of vitamin D3 and Shh may be protective of endothelial junction disruption through modulation of MP expression.

T12-36B

The Sodium Vitamin C co-Transporter-2 (SVCT2): a key molecule for microglia physiology

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Ascorbate is an antioxidant with many functions in the central nervous system (CNS) such as maturation of glutamatergic neurons and regulation of NMDA receptor. It is transported in a sodium-dependent manner by a plasma membrane transporter belonging to slc23a2 family (sodium vitamin C co-transporter-2; SVCT2). Microglia are a population of resident immune cells in the CNS. When stimulated, these cells trigger neuroinflammatory responses that may lead to neuronal cell death. Here, we demonstrate the SVCT2 expression in microglial cells, explore its importance for microglia physiology and study how pro- inflammatory stimulation of microglia (ischemia-reperfusion injury and exposure to lipopolysaccharide, LPS) regulates SVCT2 in the plasma membrane of microglia. We demonstrate by confocal microscopy, western blotting and PCR analysis that microglial cells express SVCT2. To better understand the role of this protein in microglial physiology, we performed shRNA-mediated SVCT2 Knockdown and observed that a reduction of approximately 50% in protein expression was sufficient to trigger a pro- inflammatory signature in primary retinal microglial cells, with increased ROS production, nuclear translocation of NF-kB, increased iNOS expression and increased pro-inflammatory cytokine production (measured by qRT-PCR and ELISA). Consistent with a critical role for SVCT2 in activating microglia, $SVCT2^{+/-}$ mice showed pronounced microgliosis both in the retina and cerebral cortex. To explore the role of SVCT2 in the pro-inflammatory stimulation of microglial cells, we challenged these cells by LPS intravitreous injections or by performing the ischemia- reperfusion injury in the eye, and evaluated the expression and localization of SVCT2 by high-resolution confocal microscopy. We showedin vivothat both LPS injection or ischemia-reperfusion injury in the eye trigger a robust SVCT2 downregulation in retinal microglia. We also analyzed the signaling pathways involved in this process and clearly show (by biotinylation, immunoprecipitation and FRET) that the tyrosine kinase c-Src phosphorylates caveolin-1 at tyrosine 14 leading to both caveolin-1-mediated SVCT2 internalization and SVCT2 degradation in the lysosome. To demonstrate the significance of this pathway in microglia activation we showed that LPS-induced microglia activation was abolished by overexpressing either SVCT2 or caveolin-1 phosphodefective construct (Y14F point mutation). Overall, our data demonstrate an essential role for SVCT2 in controlling the pro-inflammatory polarization of microglia and neuroinflammation.

T12-37B

Is microglial C/EBPβ deficiency neuroprotective in EAE? A new mouse model to study its implications *in vitro* and *in vivo*

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We have recently shown that the b-zip transcription factor C/EBPβ regulates proinflammatory gene expression in microglia. This suggests that microglial C/EBPβ inhibition could have therapeutic potential in CNS disorders with a pathogenic neuroinflammatory component. To test this hypothesis in animal models of neurodegenerative diseases, we have generated mice with specific microglial C/EBPβ deficiency. Mice with Cre expression under the microglial/macrophage promoter LysM, were crossed with C/EBPβ$^{fl/fl}$ mice and double mutants LysMCre C/EBPβ$^{fl/fl}$ were selected. These animals showed normal fertility, survival and organ histology in contrast to mice with full C/EBPβ deficiency. In primary microglial cultures from LysMCre C/EBPβ$^{fl/fl}$ mice, lack of C/EBPβ was observed in virtually 100% of microglial cells, whereas astrocytes showed normal C/EBPβ expression. Microglial C/EBPβ absence resulted in the almost total blockade of NO production induced by LPS+IFNγ and in altered defense response to bacteria. RNAseq transcriptome analysis of wild-type *vs* C/EBPβ-deficient microglia treated with LPS, LPS+IFNγ or vehicle revealed that the expression of 1068 microglial genes was significantly affected by C/EBPβ absence. Gene Ontology terms enrichment analysis indicated a key role of C/EBPβ in the regulation of immune and inflammatory responses. To extend these findings to the *in vivo* situation, adult mice microglia was acutely isolated from both genotypes. Microglia from LysMCre C/EBPβ$^{fl/fl}$ mice showed C/EBPβ depletion in almost 90% of cells, as well as, a marked reduction in proinflammatory genes. Finally, because C/EBPβ was markedly upregulated by experimental autoimmune encephalomyelitis (EAE) in wild-type mice, control and LysMCre C/EBPβ$^{fl/fl}$ mice were subjected to this model. LysMCre C/EBPβ$^{fl/fl}$ mice presented delayed onset and attenuated EAE severity. Altogether, these results support the hypothesis that C/EBPβ is a key regulator of proinflammatory gene expression in microglial cells and that its inhibition has an important therapeutic potential. Supported by La Marató de TV3 (110530) and Instituto de Salud Carlos III, Spain-FEDER funds, European Union (PI12/709 and PI14/302).

T12-38B

Glial activation is associated with l-DOPA induced dyskinesia and blocked by a nitric oxide synthase inhibitor in a rat model of Parkinson's disease

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l-3, 4-dihydroxyphenylalanine (l-DOPA) is the most effective treatment for Parkinson's disease but can induce debilitating abnormal involuntary movements (dyskinesia). Here we show that the development of l-DOPA-induced dyskinesia in the rat is accompanied by upregulation of an inflammatory cascade involving nitric oxide. Male Wistar rats sustained unilateral injections of 6-hydroxydopamine (6-OHDA) into the medial forebrain bundle. After three weeks animals started to receive daily treatment with l-DOPA, combined with an inhibitor of neuronal NOS (7-nitroindazole, 7-NI) or vehicle. 7-NI treatment prevented abnormal involuntary movements in l-DOPA- and vehicle-treated animals. Moreover, in l-DOPA-treated dyskinetic animals, 7-NI co-administration curtailed the increase of (i) striatal and pallidal astrogliosis, as estimated by the expression of glial fibrillary acidic protein (GFAP), (ii) CD11b-positive microglial cells with activated morphology, and (iii) inducible nitric oxide-synthase (iNOS)-immunopositive cells. These findings provide evidence that the development of l-DOPA-induced dyskinesia in the rat is associated with activation of glial cells that promote inflammatory responses. The capacity of 7-NI to prevent the glial response in l-DOPA-treated animals points to a strong involvement of nitric oxide and its synthetizing enzyme, iNOS, in the development of dyskinesia. Our observations indicate nitric oxide synthase inhibitors as a therapeutic strategy for preventing neuroinflammatory and glial components of dyskinesia pathogenesis in Parkinson's disease.

T12-39B

The effects of systemic infection on neuroinflammation in Alzheimer's disease

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Evidence from clinical studies indicates that intercurrent systemic infection, possibly mediated by inflammatory signals (e.g. cytokines), contributes to cognitive decline in Alzheimer's disease (AD) patients. Animal models suggest that microglia, 'primed' by neurodegeneration, and then activated by systemic cytokines, may augment the disease process. Here, we sought to analyse whether systemic infection in humans modifies neuroinflammation in AD.

Post-mortem neocortex of non-neurological control (ctrl) and AD patients who died with (ctrl n=18; AD n=40) or without (ctrl n=24; AD n=28) systemic infection was provided by BRAIN UK and the South West Dementia Brain Bank. Amyloid-β (Aβ, 4G8) and microglia (ionised calcium-binding adapter Iba1, phagocytic marker CD68, and Fc-gamma receptor I - CD64) were assessed by immunohistochemistry and presented as a protein load (%). In addition, different mRNA markers of microglial activation, namely IFNγ, IL1β, IL6, TNF, NOS2, COX2, TGFβ1, IL10, ARG1, YKL40, CD163 and TREM2, were measured by real-time PCR in the AD cohort with vs. without infection.

We found an effect of AD with a significant increase of Aβ (pp=0.048) and CD64 (p=0.002) load but not of Iba1 load in the AD cases (with or without systemic infection) compared to controls (with or without infection). No effect of systemic infection was observed by immunohistochemistry in either control or AD brains. In controls and AD cases, the presence of systemic infection was associated with the correlation of Aβ with CD64 (control: p=0.031; AD: p=0.043) and Iba1 with CD68 (control: p=0.019; AD: p=0.003). In AD cases with and without infection, CD64 correlated with CD68 (with infection: p=0.037; without infection, p=0.003). In addition, a significantly elevated expression of the microglial anti-inflammatory marker YKL40 (chitinase-3-like protein 1; p=0.012) was observed in the AD cases with infection vs. without infection, with no change of gene expression detected for the other inflammatory molecules.

Our current findings suggest that neuroinflammation responds differently to neurodegeneration and systemic infection. We are currently analysing phosphorylated tau and additional markers of microglia to clarify the state of microglial activation.

T12-40B

Microglial activation beyond the ganglion-cell layer in contralateral retina to experimental unilateral ocular hypertension

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Question: This study examines the effects of unilateral laser-induced ocular hypertension (OHT) in the microglia of the eyes with OHT and in their contralateral normotensive untreated eyes.

Methods: In adult albino Swiss mice unilateral OHT were induced by photocoagulation of the limbal and episcleral veins. The mice were assigned to a naive and a lasered group, the latter being analyzed for both the lasered as well as the contralateral untreated eyes. Two weeks after the

treatment the animals were killed and the retinas, as whole-mounts, were immunostained with, anti-Iba-1, anti-MHC-II, anti-CD68, anti-CD86, and anti-Ym1.

Results: In the lasered group, hypertensive as well as contralateral eyes underwent activation of the retinal microglial 15 days after the laser treatment. Specifically, a proliferative gliosis of Iba-1+ cells characterized by process retraction, an increased complexity of microglial branching, soma displacement, and reorientation of processes, were detected beyond the ganglion cell layer. In the contralateral normotensive untreated eyes, widespread gliosis similar to the hypertensive eyes occurred despite the absence of evidence of neuronal involvement. Also, microglial cells exhibited an MHC-II upregulation in OHT-eyes as well as contralateral eyes. Only eyes with OHT had rod-like microglia and rounded Iba-1+ CD68+CD86+ cells.

Conclusions: Our findings lead us to postulate that the microglial activation found in contralateral eyes could be related to neuroprotection. The implication of contralateral eyes in this model could provide new insights into glaucoma pathophysiology and may offer the potential for discovering points that could assist the development of future neuroprotective strategies for treatments.

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T12-41B

Astrocyte-targeted IL10 production modifies expression of TREM2 and CD200R in activated microglia after perforant pathway transection

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One of the endogenous mechanisms regulating inflammatory cell activation following brain injury is the expression of modulator and/or inhibitor membrane receptors. Recently, studies on endogenous regulatory receptor TREM2 demonstrate that TREM2-mediated phagocytic function of microglia/macrophages (MM) is required for debris clearance and inhibitory receptor CD200R-mediated deactivation of microglia is necessary for maintenance of Central Nervous System (CNS) tissue homeostasis. TREM2 needs an adaptor protein DAP12 to initiate the intracellular signalling cascade via an ITAM domain and tyrosine kinases. The factors that control the expression of TREM2 after CNS injury are still unclear, although the influence of the microenvironment, especially the local production of cytokines may play a key role. Hence, the objective of this study was to characterize the effects of local production of the anti-inflammatory cytokine IL-10 on TREM2 and CD200R expression using an axonal anterograde degeneration model.

For this purpose, unilateral perforant pathway transection (PPT) was performed in transgenic mice with astrocyte-targeted production of IL-10 (GFAP-IL10Tg) and their corresponding wild types (WT) littermates. At 2, 3, 7 and 14 days post-lesion (dpl) animals were perfused with 4% of paraformaldehyde and brains processed for immunohistochemistry against CD200R, TREM2 and DAP12.

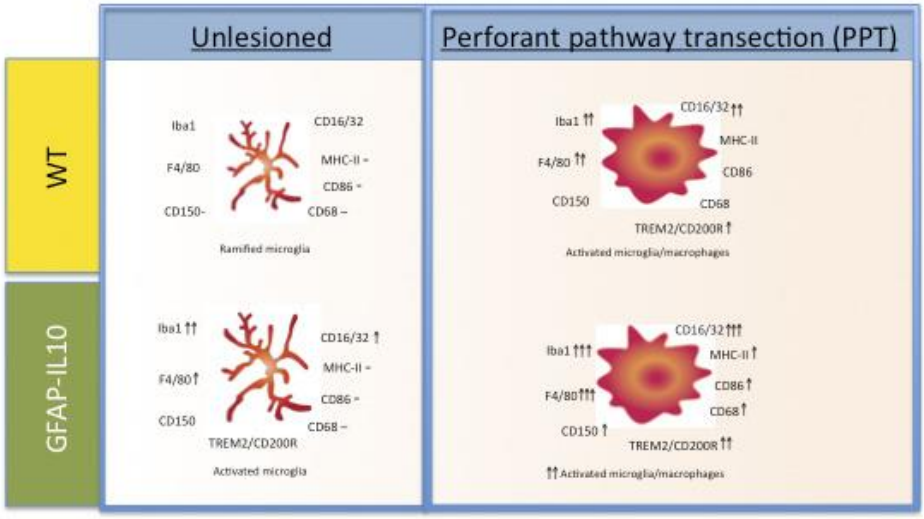
Our results showed a basal low expression of TREM2 in non-lesioned (NL) GFAP-IL10Tg animal in contrast to WT where TREM2 was mostly absent. An increase in TREM2+ MM was noted in the ipsilateral hemisphere of the GFAP-IL10Tg animal especially in the molecular layer of the dennervated dentate gyrus and was always higher than that of its respective WT. Almost all TREM2+ cells colocalized with DAP12 in both WT and GFAP-IL10Tg animals at all time-point studied. About CD200R, there was no expression in the NL WT animals; whereas a low level of CD200R was observed on NL GFAP-IL10Tg animals. After PPT, an increase of CD200R was observed in both animals but the peak of expression in GFAP-IL10Tg was delayed compared to WT.

In conclusion, this study demonstrates that local production of IL-10 by astrocytes in the CNS modifies the microglial response associated with PPT by modulating endogenous regulatory receptors as

TREM2 and CD200R. Future studies are concentrated on evaluating whether the differences of TREM2 and CD200R expression affects axonal sprouting.

Supported by Ministry of Science and Innovation (BFU2011-27400 and BFU2014-55459)

Image



T12-42B

Pathologic T cell cytokines have both beneficial and deleterious effects on oligodendrocyte lineage cells

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The acute multiple sclerosis (MS) lesion is a highly inflammatory environment characterized by demyelination and oligodendrocyte loss. A clear pathologic role for inflammatory T cell subsets (Th1 and Th17) and cytokines (IFNγ, IL-17, GM-CSF) in MS and the animal model EAE have been demonstrated. Evidence for direct cytokine- induced effects on oligodendrocyte progenitor cells (OPCs) that undertake the reparative process of remyelination has been less forthcoming. We first investigated cytokine receptor expression in isolated mouse OPCs by RT-PCR. Then OPCs were stimulated with IFNγ, IL-17, or GM-CSF and their viability, proliferation, and maturation were assessed by histological and molecular techniques in culture. The prototypical Th1 cytokine IFNγhad a deleterious effect directly inducing cell death in pure cultures of OPCs. Surprisingly the more recently identified Th17 cytokines IL-17 and GM-CSF had mixed effects. IL-17 inhibited OPC proliferation in vitro with no loss in cell viability. Rather IL-17 stimulated OPC maturation indicated by increased expression of mature myelin proteins. IL-17 also increased the myelin-specific protein, proteolipid protein (PLP), in an ex vivo cerebellar slice culture assay. Conversely GM-CSF promoted OPC proliferation in vitro also without affecting viability. Furthermore GM-CSF had no significant effect on OPC maturation in cultured OPCs nor in slice cultures. IL-17 and GM-CSF induced unique chemokine and receptor expression changes suggesting varied roles in OPC migration as well. These results suggest that inflammatory cytokines despite contributing to aberrant immune function in the lesion can have different effects on remyelination and repair. A full characterization of pro- and anti- inflammatory cytokine effects on oligodendrocytes and remyelination may provide clues for more targeted therapeutic strategies for MS.

T12-43B

Diazoxide attenuates neuroinflammation and enhances neurogenesis after NMDA-induced excitotoxicity in the rat hippocampus

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Diazoxide, an old-known mitochondrial K_{ATP} channel opener, has been proposed as an effective and safe treatment to confer neuroprotection and inhibit neuroinflammation. In this work we postulated that diazoxide attenuates the microglia inflammatory profile and modifies the neurogenesis processes associated with brain injury.

We found *in vitro* that BV2 microglia modify the expression of forty-two inflammatory molecules in response to pro-inflammatory signals and that diazoxide reverses the expression of twenty-two of these molecules. We then studied in rats the long-term effects of a daily oral administration of 1mg/kg/day diazoxide on the N-Methyl-D-Aspartate (NMDA)-induced hippocampal lesion. Diazoxide decreased the NMDA-induced neuronal loss and microglial reaction. NMDA also induced cell proliferation and neurogenesis in the lesioned non-neurogenic hippocampus detected by both doublecortin immunohistochemistry and bromo-deoxyuridine (BrdU)-NeuN double immunohistochemistry. We also found expression of the transcription factor Sp8 by cells in the injured hippocampus, but not in the intact dentate gyrus, which indicated that the lesion-induced neurogenesis involves migration of progenitors from the subventricular zone. Diazoxide treatment increased the appearance of BrdU/NeuN double stained cells and enhanced the number of Sp8-positive cells in the lesioned hippocampus. Taken together, our results indicate that diazoxide treatment attenuates the microglia inflammatory profile and enhances progenitor cell migration towards damaged areas.

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T12-44B

Microglial activation is dectected in mice retina contralateral to experimental glaucoma but rod-like microglia is restricted to eyes with ocular hypertension

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Question: To analyze qualitative changes in retinal microglia after two weeks of unilateral laser-induced ocular hypertension (OHT) both in OHT eyes and in their contralateral eyes

Methods: Adult albino Swiss mice were assigned to two groups: naïve (n=6) and OHT (n=6). Retinal whole mounts were immunolabeled with antibodies against Iba-1, MHC-II, ED-1 and NF-200.

Results: In both groups there were ramified Iba-1+ cells in the nerve-fiber layer related to the blood vessels. In OHT eyes and in contralateral eyes Iba-1+ cells had morphological signs of activation and up-regulation of MHC-II and CD68. However, only in eyes with OHT were there rod-like microglia which ran parallel to the axons and related to degenerated retinal ganglion cells (NF-200+). Rod-like microglia exhibited changes in morphology and in MHC-II and CD68 immunostaining suggestive of different levels of activation.

Conclusions: Fifteen days after unilateral OHT the microglia showed signs of activation both in OHT eyes and in contralateral eyes. Rod-like microglia was restricted to OHT eyes, the only one in which

retinal ganglion cells with signs of degeneration were found. Activated microglia in contralateral untreated normotensive eyes could exert a neuroprotective role.

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T12-45B

Structure-activity of neurostatin and other O-acetylated gangliosides as anti-inflammatory drugs on microglial cells

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Neurostatin is a natural compound purified from mammalian brain and characterized as the O-acetyl derivative in the terminal sialic acid of disialoganglioside GD1b. Neurostatin is cytostatic for astroblasts and various human and rat astrocytoma cell lines at low concentrations. Although we have previously developed different methods to synthesize Neurostatin by chemical O-acetylation of GD1b, we obtained many side products and the yield was very low. We developed a new method of synthesis of Neurostatin, using an enzyme isolated from the bacteria *Campylobacter jejuni,* with specific O-acetyltransferase activity for terminal residues of sialic acids. This enzymatic method is very specific and O-acetylates GD1b and other gangliosides in very high yield.

Here, we show that Neurostatin at a nanomolar concentration inhibits LPS-induced nitric oxide production in microglial cells, through the inhibition of NFκB. Other O-acetylated gangliosides reduced LPS-induced nitric oxide production but with less activity than Neurostatin.

This procedure opens the possibility of using Neurostatin as an anti-inflammatory agent that specifically inhibits glial cell proliferation and activation after brain or spinal cord injury.

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T12-46B

The role of microglia and inflammation in an animal model of ALS

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Background: Microglial (MG) cells activation and CNS-inflammation affect the spinal cord of $Sod1^{G93A}$ mice -i.e. the animal model of Amyotrophic Lateral Sclerosis (ALS) disease. The central role of MG cells in mediating part of the damage occurring into the CNS during neuroinflammation has been highlighted by several pioneering works. Nevertheless, the MG exact role and how neuroinflammatory episodes might influence early steps of the disease as well as motor neurons (MN) degeneration are still unresolved aspects that need further investigations.

Methods: Here we provide electrophysiological recordings of neuronal networks from WT and $Sod1^{G93A}$ mice receiving a pro-inflammatory cocktail of cytokines, that is mimicking the inflammatory

environment occurring *in vivo*. We next characterized MG cells in the spinal cord of Sod1^{G93A} mice, trying to establish a functional correlation between MG activation and neuronal damage. Finally, we attempted to polarize MG cells in Sod1^{G93A} mice toward a non-inflammatory M2 paradigm by using a gene therapy approach which is based on lentiviruses encoding the anti-inflammatory cytokine IL4.

Results: *The administration of pro-inflammatory cytokines, usually expressed by activated microglia, to neuronal networks did not perturbed their ability to fire, suggesting that young* Sod1^{G93A} neurons do not bear any functional phenotype or an increased susceptibility to the inflammatory milieu. Furthermore, *inflammatory cytokines do not perturb the bursting activity of these networks -i.e. the length of bursts, percentages of channels displaying bursting activity and the intra-burst spikes frequencies-. We next attempted to inhibit microglia activation and, as consequence, the release of pro-flammatory cues in vivo by administering* SOD1^{G93A} mice *with IL4 expressing lentiviruses*. The expression of IL4 in SOD1^{G93A} mice reduces the number of MG cells and modulates microglia-related inflammatory genes. Surprisingly, the reduction of microgliosis neither increases mice survival nor ameliorates the pathology progression, suggesting that microglia activation is only a part of the complex detrimental phenotype occurring in the CNS of ALS mice.

T12-47B

Immune system changes after adult brain injury define scar formation

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Upon traumatic brain injury the restoration of tissue architecture and function begin with the replacement of lost neurons. This is a difficult task, not only due to the restricted neurogenesis of the adult brain, but also to the exacerbated reaction of glial cells during the regenerative process. The glial scar is the hallmark of the reaction to injury in the mammalian brain and it is well known to create a very “hostile” environment that tremendously reduces neural survival and integration. Therefore, understanding glial scar formation is the first step in modulating the scarring process in order to promote functional recovery.

To study glial scar formation, we use an animal model with great regenerative potential even in adulthood; the zebrafish. We have established two types of stab wound injuries in the zebrafish telencephalon with different scarring processes, a “scarless” injury (nostril injury) (Kroehne et al., 2011; Baumgart et al., 2012) and the skull injury (März et al., 2011) with Olig2-GFP$^+$ cells and microglia forming a scar similar to what is observed in the mammalian system. Importantly, the scar formed by the Olig2$^+$ cells remains in the regenerating brain for at least 4 weeks. The characterization of these two types of injuries shows major differences in the microglia and oligodendrocyte progenitors (OPCs) activation. Microglia react strongly after both injuries, but the morphology, distribution and kinetics of activation of 4C4$^+$ microglia differs greatly between the two paradigms, suggesting a prime role of 4C4$^+$ microglia in inducing glial reactivity.

To identify the molecular mechanisms underlying the different scarring processes we performed a transcriptome analysis after both types of injury at 1, 2, 3 and 7 days post-injury (dpi). Strikingly, the molecular signature during the scar formation (skull 3 dpi) is radically different compared to the scarless injury. In this comparative microarray analysis key pathways related to immune cell signaling were identified and the latest results will be presented.

Our data suggest that the modulation of the immune response might be the key to achieve the scarless regeneration and therefore, potentially better survival of newly generated neurons.

T12-48B

Multiple sclerosis patient’s lymphocytes crosstalk with microglial cells impacts the remyelination process

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C. Sanson*, M. El Behi *, C. Bachelin, L. Guillot-Nöel, N. Sarrazin, B. Stankoff, E. Maillart, I. Rebeix, B. Fontaine #, V. Zujovic #. ICM-A-IHU, ICM, UPMC 06 UM 75, INSERM U 1127, CNRS UMR 7225, 47 boulevard de l'Hôpital, 75013 Paris, France. Objective : Myelin is essential for normal nerve conduction and its destruction leads to irreversible neurological disorders in patients suffering from multiple sclerosis (MS). An interesting feature of MS is that an endogenous remyelination process can be triggered. This process involves the recruitement and differentiation of oligodendrocyte precursor cells (OPCs) but also the participation of the Schwann cells (SC), the myelinating cells of the peripheral nervous system. The microglia cells (MIG) polarization state is of critical importance for the success or failure of remyelination and their polarization state is highly dependant of their dialogue with T helper lymphocytes (LTh). In this study we asked whether controls or MS patients LTh influence differentially MIG polarization and how it impacts the remyelination process. Methods : We determine LTh composition in the blood of healthy donors and MS patients using flow cytometry and we assess their secretory profile after their in vitro activation by luminex. We study in vitro the influence of LTh supernatants on MIG polarization towards a “classically activated” (M1) or an “alternatively activated” (M2) state; and its consequences on OPCs differentiation. We also establish a new in vivo model of demyelination by combining lysolecithine induced demyelination and human LTh grafting in the Nude mice spinal cord. So, we test the influence of controls and MS patients LTh on the remyelination process, that normally occurs in this model. Results : While LTh composition is similar in MS patients and controls blood, we evidence different molecular profiles highhlighting four cytokines differentially expressed in MS patients LTh supernatants. Moreover, MS patients LTh supernatants compared to controls LTh induce an increase of the M1/M2 microglia ratio which in turn results, in vitro, in a decrease of OPC differentiation into mature oligodendrocytes. After verifying the survival and integration of human LTh graft in the demyelinated lesion of Nude mice spinal cord, we clearly demonstrate that SC remyelination is significantly decreased in mice grafted with MS patients’ LTh. Interpretation : In conclusion, we evidence for the first time that MS patients LTh can directly influence the lesionned environment by modulating MIG polarization and impeding the repair process. Financial Support: Prix Bouvet- Labruyère, OCIRP, ARSEP, « Investissement d’avenir » ANR-10-IAIHU-06.

T12-49B

An age-specific intravascular macrophage population is associated with the murine window of susceptibility to CNS inflammation

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Perinatal inflammation can have a profound effect on postnatal development, but the characteristics of the central nervous system (CNS) response to proinflammatory challenges changes rapidly in the immediate postnatal period. However, it remains unclear what factors are responsible for the variation in the response. Here, we sought to explore the mechanisms by generating focal inflammatory lesions in the striatum with IL-1β at P7, P14, P21 and 2 months. Animals were killed at 4h and immunohistochemistry and qRT-PCR analysis were performed to explore the molecular and cellular responses.

The cellular response to the inflammatory injury was greatest at P14 and was characterized by the marked recruitment of neutrophils and monocytes, which was absent at the other time points, and blood-brain barrier breakdown was also a feature. However, the molecular response to the proinflammatory challenge was greatest at P21, when transcripts for proinflammatory cytokines, and adhesion molecules were highly unregulated compared to the other time points. Thus, these results reveal a prominent and unexpected dissociation of the molecular from the cellular events.

In search of an explanation, we have discovered that a unique population of IBA-1 positive macrophages is present in the naïve brain of P14 animals that are absent at all other time points. These cells are also ICAM-1 positive and may thus provide a nexus for further leukocyte recruitment at

this time point. Targeting this cellular population may represent a target to intervene in the leukocyte-mediated damage that can occur during the window of susceptibility.

T12-50B

Characterization of the Wnt signalling pathway in the hippocampus of mice with experimental autoimmune encephalomyelitis

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In multiple sclerosis (MS), a chronic immune-mediated disease of the central nervous system (CNS), clinical presentations of cognitive deficit have been recently correlated with hippocampal demyelination and loss of synaptic activity. Whether hippocampal neurogenesis is altered in MS patients remains to be investigated. Here, we analysed the impact of experimental autoimmune neuroinflammation on hippocampal neurogenic processes and the activity of Wnt signalling, its key regulatory pathway. Using the active mouse model of experimental autoimmune encephalomyelitis (EAE) in Axin2-LacZ reporter mice, we revealed an increase in hippocampal Wnt activity in the acute phase of disease and a correlation to its severity. These changes were associated with activation of β-catenin (β-Cat) and deactivation of glycogen synthase kinase-3β (GSK-3β), both downstream regulators of the Wnt cascade. Furthermore, we observed in the same hippocampal samples synaptic loss (PSD-95 protein expression) and neuronal degradation (neurofilament M protein expression). To further support our findings, the passive EAE model was induced by adoptive transfer of myelin-specific T cells and hippocampi of diseased animals were analysed. In the acute phase, the Wnt pathway up-regulation was confirmed by detecting transcriptional activation of Wnt-target genes (LEF1, TCF4 and Axin2) and several Wnt ligands, pathway triggering molecules. Wnt signalling activation was also associated with elevated neuroinflammation (IFN-α, TNF-α and TGF-β1) and strong microglia activation (Iba1 expression). Additionally, histological analysis in EAE mice showed enhanced proliferation (BrdU+-retained cells) and generation of neuronal progenitors (doublecortin+ neuroblasts) in the dentate gyrus, both in line with up-regulated Wnt/β-catenin signalling in the acute phase. Finally, by using organotypic hippocampal slice cultures from Axin2-LacZ reporter mice, we were able to analyse the causal connection between neuroinflammation, neurognesesis and Wnt signalling activity. Taken together, our data implicate Wnt signalling in neural response to hippocampal tissue damage and its role in generation of new-born neurons upon neuroinflammation.

T12-51B

Non-lytic autoantibody mediated injury induces chemokine expression in myelinating cultures

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Intrathecal antibody synthesis is the most consistent immunological abnormality associated with multiple sclerosis (MS). It is already established in >90% of patients at diagnosis, persists throughout the course of the disease and manifests itself as discrete oligoclonal bands of IgG in cerebrospinal fluid (CSF), but its pathophysiological significance remains obscure. This intrathecal response may recognise any of a wide variety of microbial antigens, as well as myelin, axonal and neuronal autoantigens; a complex and heterogeneous spectrum of specificities that varies between patients. Nonetheless a small, but consistent body of evidence demonstrates this disease-associated response contains a significant component directed against myelin-derived lipids, in particular galactosyl ceramide and sulphatide. These lipids are exposed at the outer surface of the myelin sheath where they provide targets for antibody-dependent, complement-mediated demyelination in a variety of experimental settings. But is this really the case in MS, in particular in progressive forms of the disease in which the blood brain barrier is intact? In this setting serum complement is unable to access the CNS, an observation that led us to explore the possibility these glycolipid-specific antibodies might play some other role in disease pathogenesis. This was investigated using myelinating cultures derived from embryonic rat spinal cord; a model system that allowed us to explore the effects of a sulphatide reactive IgM mAb O4 in the presence or absence of serum as an exogenous source of

complement. In the absence of serum, O4 was unable to induce demyelination, but inhibited ongoing myelination; an effect associated with microglial proliferation and activation. Gene microarray analysis revealed this was associated with the rapid induction of multiple chemokines. Subsequent experiments confirmed this resulted in increased protein synthesis for selected candidates and secretion of biologically active products. This glycolipid specific antibody therefore mediates a complex non-lytic response associated with induction of chemokines implicated in recruitment of T cells (CCL5, CXCL11), B cells (CXCL13, 10 and 9) and monocytes/macrophages (CCL2, CCL7) into the CNS; a response predicted to exacerbate disease activity in MS.

T12-52B

Doxycycline decreases the inflammatory response of LPS-treated microglial cells

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We previously demonstrated that the antibacterial drug doxycycline confers neuroprotection in a Parkinson disease mouse model by restraining glial cell activation (Lazzarini et al, Glia, 2013). In the present study, we wished to further explore the potential of doxycycline against neuroinflammation, using microglial cell-enriched cultures prepared from post-natal (P1) mouse brains. More specifically, we evaluated the impact that a pretreatment with doxycycline exerts on microglial cells exposed to the bacterial inflammogen lipopolysaccharide (LPS, 1 or 10 ng/ml) for 24 hours. Our results show that the calcium-binding protein Iba-1, a marker of microglial cell activation, was highly increased in Mac-1+ cells in LPS-treated cultures and that this effect was attenuated by doxycycline pretreatment in a dose-dependent manner. Moreover, in LPS-treated microglial cultures, doxycycline also diminished the production and release of two pro-inflammatory cytokines (TNF-α and IL-1β) and that of nitric oxide, a gaseous mediator of neuroinflammatory responses. In addition, doxycycline was found to effectively decrease LPS-induced intracellular radical oxygen species (iROS) production. Finally, we investigated the possible mechanisms through which doxycycline exert its modulatory effect on key pro-inflammatory markers. We found that doxycycline significantly decreases p38 phosphorylation and NF-kB nuclear translocation in LPS-treated microglial cultures, suggesting that these signaling pathways are involved in doxycycline's anti-inflammatory properties. Taken together, these results suggest that doxycycline could operate as an efficient protective agent in neuroinflammation by repressing the pro-inflammatory response of microglial cells through p38 and NF-kB signaling pathways.

T12-54B

Fibronectin aggregates maintain a mixed activation phenotype of microglia and macrophages that impairs differentiation of oligodendrocytes

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Question: Promoting endogenous remyelination in multiple sclerosis (MS) benefits from insights into inhibitory molecules that impede remyelination. Fibronectin (Fn) assembles into aggregates in MS, which impairs oligodendrocyte differentiation and remyelination. However, successful remyelination also requires the presence of microglia and macrophages, which normally switch from an M1 pro-inflammatory phenotype upon demyelination to a supportive, M2 phenotype during remyelination.

Here, we investigated whether Fn aggregates impair remyelination by modulating the phenotype of microglia and macrophages.

Methods: Primary rat microglia and bone marrow-derived macrophages were exposed to plasma Fn or deoxycholate-insoluble Fn aggregates, isolated from the astrocyte-deposited extracellular matrix. The effects on microglia/macrophage behavior and phenotype were compared to interferon-γ (IFN-γ)- or interleukin-4 (IL-4)-treated cells, representing the pro-inflammatory (M1) and anti-inflammatory (M2) phenotypes, respectively.

Results: In line with IFN-γ treatment, exposure to both plasma Fn and astrocyte-derived Fn aggregates induced an amoeboid morphology of microglia and macrophages and stimulated macrophage phagocytosis rates. However, at these conditions proliferation of microglia and macrophages was similar as that observed for IL-4 stimulated cells. Remarkably, in the presence of (astrocyte-derived) Fn aggregates, but not dimeric plasma Fn, the expression of the M1 phenotype marker iNOS, the corresponding production of nitric oxide *and* the activity of the M2-marker arginase-1 was increased. Conditioned medium derived from the Fn aggregate-induced mixed phenotype macrophages, reduced the number of MBP-positive cells, indicating that oligodendrocyte differentiation was perturbed. This was not observed with conditioned-medium obtained from macrophages grown on plasma Fn, indicating differences in the effect of non-aggregated (plasma) Fn versus aggregated Fn on macrophage phenotype and oligodendrocyte differentiation.

Conclusions: Fn aggregates, but not (dimeric) plasma Fn, promote a mixed M1/M2 macrophage phenotype that impairs the differentiation of oligodendrocytes. Thus the persistent presence of Fn aggregates in MS lesions results in a dominant M1 over M2 phenotype of microglia and macrophages, thereby creating an environment that impedes remyelination.

T12-55B

Prenatal stress causes prolonged microglial activation and enhanced inflammatory processes in the rat brain

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Recent data suggest that early life adverse experience causing alteration in the intrauterine environment may lead to the onset of depression in adult life. Among them, stressful events during pregnancy, a critical period for the development of the central nervous system, seem to be one of the most potent factor influencing brain homeostasis especially the brain's immune system. The primary immune cells in the brain are microglia, cells also considered as the main source of the cytokines in the brain. The excessive microglial activation has been postulated to play a key role in the pathogenesis of depression.

Therefore, the aim of our study was to determine whether prenatal stress procedure (an animal model of depression) may affect brain's inflammatory status and if this process is long-lasting.

Pregnant Sprague-Dawley rats were subjected daily to three stress sessions from 14th day of pregnancy until delivery. Control pregnant females were left undisturbed in their homecages. In both young 7-day-old and adult 3-month-old male offspring of control and prenatally stressed animals the expression of pro-inflammatory cytokines was evaluated. Moreover, the mRNA expression of microglial cells markers (CD68, CD40) as well as the Iba-1 protein level in the hippocampus and frontal cortex were measured. Additionally, in adult rats behavioral verification was conducted using forced swimming test and sucorse preference test.

We found that adult animals subjected to prenatal stress procedure exhibit depressive-like behaviors. Biochemical analysis demonstrated an increased level of TNF-α, IL-1β and IL-6 in the hippocampus and frontal cortex of young and adult prenatally stressed animals. Interestingly, the enhanced production of pro-inflammatory cytokines after prenatal stress procedure was accompanied by augmented activity of microglial cells, confirmed by elevated expression of CD40, CD68 and enhanced Iba-1 protein level in both young and adult rats.

In summary, our data show that prenatal stress may lead to increased and prolonged inflammatory status in the brain. Observed changes are connected with increased microglial activation, supporting the hypothesis that these cells are involved in the pathogenesis of depression.

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T12-56B

Can embryonic microglia bridge the gap between maternal immune activation and neuropsychiatric disorders?

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Several studies have indicated that inflammation during pregnancy increases the risk for the development of neuropsychiatric disorders like autism and schizophrenia in the offspring. Morphological brain abnormalities and deviations in immunity can be observed in patients of both disorders. It has been suggested that the acute infection induces changes in maternal cytokine levels which in turn affects the fetal brain and results in the development of both neuropsychiatric disorders in the offspring.

In this study, the poly (I:C) model was used to mimic viral immune activation in pregnant mice in order to study the fetal microglial response to the maternal infection. We injected pregnant mice with poly (I:C) (i.p., 20 mg/kg) on either E11.5 or E15.5. The concentration of IL-6 in the maternal serum was used as a measure for systemic inflammation in the mother. Afterwards, the microglial cell density and activation level (Mac-2, iNOS and IL1β immunostainings) in the cortex and hippocampus of CX3CR1-eGFP +/- embryos was determined. Additionally, to test the possibility that microglia are primed by a first injection and activated only upon a secondary stimulation, pregnant mice were injected with poly (I:C) on both E11.5 and E15.5.

Despite the presence of a systemic inflammation in the pregnant mice, we found no significant difference in fetal microglial expression of activation markers Mac-2, iNOS and IL1β between the control and inflammation group. These results suggest that fetal microglia are not 'classically' activated by the maternal immune activation.

T12-57B

Specific downregulation of RhoA triggers microglia pro-inflammatory signature via Rock2/Csk/c-Src signaling pathway

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Overactivation of microglia, the resident immune cells of the central nervous system (CNS), might actively contribute to progressive neuronal damage, and modulation of their activation has been suggested as a potential therapeutic target for alleviating neurodegeneration. Here, we report essential and specific functions for the small rhoGTPase RhoA in regulating microglia physiology. We

show that knocking down the expression of RhoA, but not that of RhoB or RhoC, in cortical microglia is sufficient to trigger the transition from a resting-like phenotype into an amoeboid cell morphology, increase ROS production and glutamate release, which causes neuronal loss in cultures from embryonic neurons. Intramolecular FRET biosensors to specifically monitor RhoA or RhoC activity indicated that inflammatory stimuli (LPS or hypoxia) rapidly downregulate RhoA activation while these same insults were inefficient in altering RhoC activity in cortical microglia. Knocking out RhoA using CRE-mediated recombination in microglia obtained from RhoA floxed mice promoted cell shape changes, acquisition of a pro-inflammatory spectrum, increased ROS generation and glutamate release that could neither be reproduced by knocking down RhoB/RhoC nor compensated by an upregulation of RhoC activation. Furthermore, knocking down Rock2, but not Rock1, mimicked all the aspects of RhoA ablation in microglia and further reduced the expression of the endogenous repressor of the c-Src tyrosine kinase, Csk. These results show that RhoA/Rock2/Csk signaling regulate microglia activation. Accordingly, the genetic or pharmacological inhibition of c-Src prevented the acquisition of a pro-inflammatory microglia signature, ROS production and glutamate release in RhoA, Rock2, and Csk-mediated microglia activation. Overall, we provide novel mechanistic insight into how RhoA-mediated signaling pathways regulate microglia function. Such knowledge might help paving the way for more efficient strategies for controlling microglia overactivation.

T12-58B

Modulation of neuroinflammation by the microglial inhibitory receptor CD200R1

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In the healthy CNS, microglial reactivity is prevented by the presence of an inhibitory environment where neuronal inhibitory signals play a relevant role. In response to noxious stimuli microglia develop reactive phenotypes, characterized by the production of pro-inflammatory factors with potential neurotoxic effects, suggesting that the inhibitory mechanisms have been overcome. We focused our interest on the study of one of these inhibitory mechanisms, CD200 ligand (mainly neuronal but also present in astrocytes) - CD200R1 receptor (microglial) interaction. Alterations in CD200 and CD200R1 expression have been described in neurological diseases, suggesting that inhibitory mechanisms are modified in pathological conditions.The aim of the present work was to study the effect of CD200R1 modulation on microglia reactive phenotype using experimental *in vitro* and *in vivo* approaches.

Glial cell cultures were treated with a pro-inflammatory stimulus (LPS+IFN-γ) and the pattern of expression of pro- and anti-inflammatory molecules was determined after CD200R1 activation and CD200R1 inhibition. The induction of the expression of pro-inflammatory molecules was inhibited in reactive primary microglial cells treated by a CD200R1 agonist, while the expression of anti-inflammatory molecules was enhanced. Inhibition of CD200-CD200R1 interaction in mixed glial cultures using a blocking antibody potentiated the pro-inflammatory response. Finally, mice were treated with an i.p. dose of LPS and the effect of CD200R1 activation on the expression of pro- and anti-inflammatory molecules in isolated adult microglial cells was determined. We observed that LPS induction of pro-inflammatory markers was inhibited by CD200R1 agonist treatment, and that the induction or inhibition of anti-inflammatory markers was attenuated.

These results show that the reactive phenotype of glial cells can be modulated through an action on CD200R1, suggesting CD200R1 as a candidate target to act against neuroinflammation in neurodegenerative diseases.

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T12-59B

Anti-inflammatory therapy via CD163-macrophages in the 6-OHDA Parkinson's disease model

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Parkinson's disease (PD) is characterized by the progressive degeneration of dopaminergic neurons in the substantia nigra (SN) and the presence in cells of aggregated alpha-synuclein (a-syn) in Lewy bodies. Among other factors, inflammation seems to play a role in PD neurodegeneration. We have data suggesting infiltration of peripheral immune cells, specifically CD163+ macrophages, into the area of neurodegeneration in the 6-hydroxydopamine (6-OHDA) PD model. We hypothesized that the migration of CD163+ macrophages into the brain-injured area in PD may influence local microglia. By enhancing M2 profile of peripheral macrophages, these could in turn release anti-inflammatory molecules into the neurodegenerative area modifying local microglia response and resulting in neuroprotection. In a 6-OHDA rat model of PD, designed liposomes targeted for the scavenger receptor CD163 were used to deliver dexamethasone (Dexa) into peripheral macrophages. The liposomes were injected intravenously (i.v) for 3 weeks in a treatment approach, with in parallel 3 control groups. Our data show that liposomes Dexa loaded CD163+ macrophages were able to reach the brain. The treatment modified the peripheral immune system and induced an improvement of the motor functions. This paralleled a partial rescue of dopaminergic neurons in the nigro-striatal system. Injection of free Dexa resulted in significant overall weight loss, decrease in thymus weight and endogenous cortisol level in serum and those side effects of Dexa were not observed in animals treated with Dexa-loaded liposomes. Our data support the modulation of inflammation by targeting macrophages as a putative novel therapy in PD and the use of CD163 targeting a valid and non-invasive approach to modulate neuroinflammation in neurodegenerative diseases.

T12-60B

Anti-VLA-4 treatment reduces microglial activation in a focal EAE-model

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Question: Anti-VLA-4 is one of the most effective treatments for multiple sclerosis (MS) (Hutchinson et al., 2009). The anti-VLA-4 monoclonal antibody (mAb) binds to the α4-chain of α4β1-integrins, also called very late activating antigen4 (VLA-4), which are expressed on all leukocytes excluding neutrophils. It inhibits leukocyte binding to vascular cell adhesion molecules (VCAM-1) and fibronectin and, thus, inhibits lymphocyte infiltration into tissues (Vosoughi and Freedman, 2010). The aim of this study was to evaluate the effect of anti-VLA-4 treatment on microglial activation in type 1 focal EAE rodent model of MS.

Methods: Focal EAE was induced by a stereotaxic injection of BCG into the striatum, and by subsequent peripheral activation of the lesion using complete Freund's adjuvant. Subcutaneous treatment with anti-VLA-4 mAb was initiated 30 days after the peripheral activation of the lesion, and continued for 14 days in 4 rats. Control rats (n=4) were treated with an isotype matched non-binding control antibody. The animals were imaged in vivo using [^{18}F]-GE-180 and positron emission tomography (PET) in the beginning and after 14 days of the treatment to evaluate the effect of the drug on lesion development. [^{18}F]-GE-180 is a radioligand that binds to the 18 kDa translocator protein expressed on activated microglia. The results were confirmed by immunohistochemistry by staining the microglia with anti-OX-42 mAb.

Results: In vivo PET imaging demonstrated a significant beneficial effect of the treatment, which was detected by reduced binding of the radioligand [^{18}F]-GE-180, and interpreted as a significant reduction in microglial activation following treatment. The lesion-to-contra ratio binding was reduced after 14 days of treatment when compared to the control group ($p = 0,036$). Analysis of histology confirmed the reduction of microglial activity in the treated animals compared to the control animals.

Conclusions: Our study demonstrates that anti-VLA-4 treatment reduces the inflammatory reaction and microglial activation in the focal EAE model. Importantly, this can be measured using in vivo TSPO PET imaging.

T12-61B

CD200-CD200R1 system in multiple sclerosis

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Multiple sclerosis (MS) is a complex neurological disease that affects more than 2.3 million people worldwide. MS is an inflammatory demyelinating disease of the central nervous system (CNS), although its etiology is unknown. Microglia play an important role in neurological diseases as executer of innate immunity to restore tissue homeostasis and minimize neuronal damage. However, the exacerbation of microglial activation seems to play a negative role in disease progression, since pro-inflammatory factors overproduction can contribute to neuronal death. Neurons have developed several inhibitory mechanisms to control microglia activation, such as CD200 (neuronal)-CD200R1 (microglial) interaction. Neuron-microglia interaction could be a therapeutic target to control glial activation/neuroinflammation, however little is known about the mechanisms that regulate this interaction and very few studies are performed in human. In the present work, we studied the expression of CD200-CD200R1 system in an experimental model of MS (EAE, experimental autoimmune encephalomyelitis) as well as in postmortem brain samples from multiple sclerosis patients and controls. In the EAE model, CD200R1 expression was increased in spinal cord at symptomatic disease phase, while CD200 expression was decreased both at pre-symptomatic and symptomatic phases. Interestingly, CD200R1 and CD200 expression were also altered in the brain. In MS samples, CD200R1 expression was noticeable increased at both mRNA and protein levels. In contrast, we observed a decrease in CD200 expression in these samples. Therefore, CD200-CD200R1 system is differentially expressed during development and progression of demyelinating diseases, which suggests that modulation of CD200-CD200R1 could be a good tool to control microglial activation in neurological demyelinating diseases and minimize the chronic inflammatory response associated with multiple sclerosis.

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T12-62B

Liver X receptor beta deficiency decreases neuroinflammation in an animal model of multiple sclerosis

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Liver X receptors (LXRs) are master regulators of cholesterol turnover and inflammatory responses. Activation of LXRs by a synthetic agonist in an animal model of multiple sclerosis, experimental autoimmune encephalomyelitis (EAE), was shown to suppress autoreactive T cell responses and subsequent disease symptoms. In this study, we determined the role of the LXR isoforms on the onset of neuroinflammation by inducing EAE in LXRalpha and LXRbeta deficient mice. Interestingly, while disease severity was not significantly affected in LXRalpha deficient mice, disease symptoms were significantly reduced in mice lacking LXRbeta. A bone marrow transplantation and an adoptive T cell transfer revealed that the reduced disease severity was not due to the absence of LXRbeta in peripheral immune cells. At disease onset immune cell infiltrates were significantly reduced in the

central nervous system (CNS) of LXRbeta deficient mice, which coincided with a reduction in spinal cord chemokine expression. *In vitro*, activation of LXRs by the LXR agonist GW3965 induced microglial chemokine expression that was not detected in endothelial and astroglial cells. These findings indicate that LXRbeta controls immune cell infiltration into the CNS by inducing chemokine expression in microglia. Future studies should elucidate the cell type and disease process specific function of LXRalpha and LXRbeta for optimal targeting of these nuclear receptors.

T12-63B

Induction of microglia M2 polarization in male and female mice and in response to estrogens using icv injection of IL4

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Microglia have the unique property to sense any pathological event and to immediately undergo biochemical and morphological transformations that destroy the damaging insult and trigger tissue repair. The alternatively activated M2 phenotype is associated with anti-inflammatory and regenerative activities, although the ability of microglia within specific brain regions to respond to polarizing signals is not understood yet. We have since long been interested in studying the neuroimmunomodulatory activity of the female steroid hormones, estrogens, and analyzing their role in the sexual dimorphism observed in the pathophysiology of selected neurodegenerative and inflammatory diseases. We previously showed that estrogens can reduce the M1 pro-inflammaotry response of microglia in vivo and in vitro. The aim of the present study is thus to optimize an animal model of M2 polarization by using intracerebroventricular (icv) injections of interleukin-4 (IL4)[1] and to compare microglia polarization among males and females and in response to circulating estrogen levels. The expression of M2 genes and proteins was evaluated in mouse brain areas at different time points following icv IL-4. Our data show that only a subpopulation of microglia is able to respond to icv IL-4 which may translate into physiologically and pathologically relevant region-specific differences in the regenerative potential of microglia against brain insults. Our preliminary results also show a sex and estrogen-dependent dimorphism of microglia responsiveness to M2 activating signals, suggesting a relevant role for this hormone on microglia reactivity in acute and chronic neuroinflammation.

[1]Pepe G. et al, J Neuroinflammation 2014

T12-64B

Differential expression of TREM2 in transgenic mice with CNS-targeted IL-6 or IL-10 production correlates with opposing effects on neurodegeneration after facial nerve axotomy

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Question: After facial nerve injury in the mouse, some facial motor neurons die and an immune response occurs with broad microglial activation and T cell infiltration within the lesioned nucleus. The main functions of activated microglia are to modulate the inflammatory response (through the secretion of proinflammatory cytokines) and to phagocytose dying cells. Whether phagocytosis worsens or improves neuronal survival remains unknown. In this regard, phagocytic microglia with neuroprotective features have been associated with an increase in triggering receptor expressed on myeloid cells-2 (TREM2). Cytokines, such as interleukin-6 (IL-6) and interleukin-10 (IL-10), may impact TREM2 expression and thus, control the balance between noxious and beneficial phagocytic microglia. The objective of the present study was to evaluate the effects of local production of either IL-6 or IL-10 on neuronal degeneration and microglial phagocytosis induced by axotomy of the facial nerve.

Methods:To accomplish this, we used two transgenic mice, GFAP-IL6Tg and GFAP-IL10Tg, which express either the murine IL-6 or IL-10 gene under the transcriptional control of the GFAP promoter in astrocytes. Unilateral facial nerve resection was performed on these transgenic mice and their corresponding wild-type (WT) littermates. Animals were euthanized at 3, 7, 14, 21 and 28 dpi, and cryostat free-floating sections were processed for immunohistochemical analysis.

Results: Our results showed that IL-6 production had a detrimental effect on neuronal survival, whereas, IL-10 production was able to protect against neuronal death. These changes in neurodegeneration correlated with differential TREM2 expression observed between the two transgenic animals: GFAP-IL10Tg mice had higher levels of TREM2 expression at 21 dpi; while in GFAP-IL6Tg mice, these levels were significantly lower at this time-point. We showed that most TREM2 + cells co-localized with Iba-1, a pan marker for microglia, and CD16/32; and a subpopulation of these TREM2+ cells also expressed CD68.

Conclusions:In this study we showed that astrocyte-targeted IL-10 and IL-6 production have a direct impact on neuronal survival, and modify microglial phagocytic function; leading to opposing outcomes after facial nerve axotomy.

T12-65B

Multiplexed synchrotron X-Ray fluorescence imaging of brain inflammation using targeted heavy metal nanoparticles

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Acute inflammatory disease in the CNS is often associated with increased permeability of the blood-brain-barrier (BBB) and recruitment of leukocytes to focal lesions. However, these events, which can be probed with conventional structural imaging techniques, often fail to reveal the true extent of disease. Several diseases like multiple-sclerosis (MS) and ischemic stroke would greatly benefit from a method that could visualize the early-stage and late-stage pathology when the BBB is intact. The use of targeted molecular imaging to explore the pathogenesis of brain inflammation provides a powerful tool for the diagnosis and assessment of downstream treatment.

Synchrotron X-Ray Fluorescence (XRF) can image changes in elemental composition of trace metals in brain tissue and reveal metabolic and physiological alterations during disease progression.[1] XRF analysis of the brain allows us to multiplex targeted intravascular contrast agents, which can be studied while simultaneously imaging biological events in the pathological progress.[2] Here we present a map of early stages of brain inflammation, prior to BBB breakage, using two distinct markers for early brain inflammation conjugated to nanoparticles of non-biological heavy elements. The distinct XRF emission lines of these elements enable us to selectively image the targeted nanoparticles during inflammation and reveal a district spread of inflammatory markers.

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T12-66B

Interleukin-33 is synthesized in response to the CNS injury to affect the response of microglia and macrophages

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Interleukin-33 is a novel protein enhancing Th2 cytokine production, initially described as an alarmin which is released from dying, necrotic cells to affect the immune response. However, more recent studies suggest active production of IL-33 and its release in the injury activated tissues. Nevertheless, synthesis and secretion of IL-33 as a cytokine in the injured central nervous system (CNS) has not been comprehensivelyelucidated so far. Therefore, the aim of the present study was to examine the potential role of IL-33 as a cytokine released in response to CNS injury. Using qPCR, Western blotting and ELISA assay we showed significant up-regulation of IL-33 mRNA and protein in two pathological models: in a response to demyelination of the spinal cord white matter and stab wound injury of the brain cortex. Using i*n vitro* cultures of activated glial cells we examined their potential to secrete IL-33. Moreover, we demonstrated that injury leads to increase in the number of IL-33 receptor (Il1rl1)-expressing $CD11b^{+}$myeloid cells, both in the CNS parenchyma and in the peripheral blood. Our results suggest that interleukin-33 functions not only as an alarmin but it is actively synthesized and potentially released to directly affect response of microglia and macrophages.

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T12-67B

Characterization of the cytokine secretion profile of highly purified, activated astrocytes

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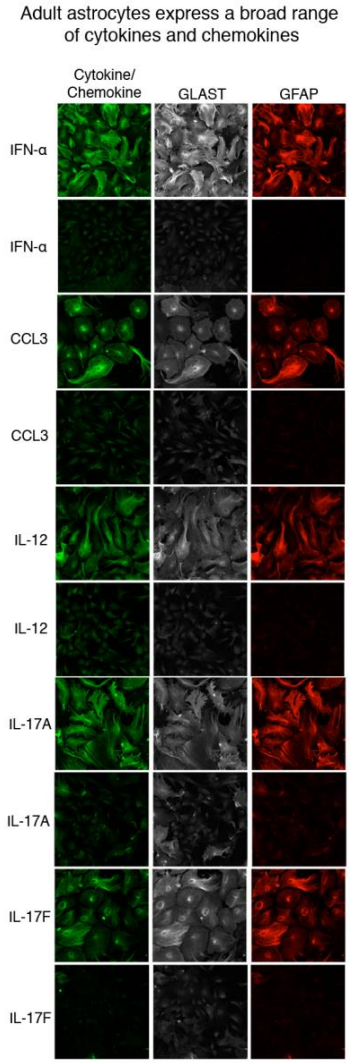
The inflammatory response in the central nervous system (CNS) in multiple sclerosis (MS) is a dynamic multifactorial process that involves both the local resident immune cells, such as microglial cells and astrocytes, as well as infiltrating circulating immune cells such as macrophages. These immune cells communicate to each other by secreting different cytokines, chemokines, and growth factors, regulating and initiating their activities. Astrocytes, a population of CNS-resident cells, were found to play multiple roles in neuroinflammation and neurodegeneration. However, there are no detailed and reproducible studies of cytokine and chemokine secretion, partly due to technical restrictions. Most astrocyte populations are not purified effectively and contain contaminating microglial cells, which can lead to erroneous interpretation of the results.

In this study, the astrocyte-specific Anti-ACSA-2 (astrocyte cell surface antigen-2) MicroBeads were used for magnetic isolation (MACS® Technology) of astrocytes from neonatal and adult mouse brain tissue. Separated cells were stained with an Anti-ACSA-2 antibody and Anti-CD11b antibody for flow cytometry analysis (MACSQuant® Analyzer) to determine astrocyte purity. Highly pure neonatal astrocytes (about 96%) and adult astrocytes (about 94%) were obtained, with very little microglial cell contamination (about 0.05%). Immunocytochemistry (ICC) showed that the cultured neonatal and adult astrocytes express a broad range of cytokines and chemokines. Lipopolysaccharides (LPS) or conditioned media from LPS-stimulated microglia were used to stimulate neonatal astrocyte culture, and supernatants were collected and analyzed by a MACSPlex Cytokine Kit (Miltenyi Biotec) to investigate cytokine secretion. A significantly increased level of GM-CSF was observed when neonatal astrocytes were treated with conditioned media from LPS-stimulated microglia or high concentration of LPS, whereas no significant change in the TNF-α level was found. This result is consistent with studies in which astrocytes were treated with L-leucine methyl ester (LME) to get highly enriched astrocyte cultures.

In conclusion, Immunomagnetic separation of mouse brain cells with Anti-ACSA-2 MicroBeads allows the isolation of neonatal and adult astrocytes to high purities. The purified cells can be cultured and used for characterization of the cytokine and chemokine secretion profiles, which was shown to express a broad range of cytokines and chemokines *in vitro*.

Image



T12-68B

The role of CC chemokine ligand 3 (CCL3) in a mouse diabetic neuropathy - *in vivo* and *in vitro* studies

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The pathomechanisms involved in diabetic neuropathic pain development is poorly understood. Currently, the role of glia parallel with the chemokines participation seems to play a crucial role in nociception. The goal of our study was to determine whether the chemokine (c-c motif) ligand 3 (CCL3) influence nociceptive transmission, is changed in diabetic neuropathy and what is its cellular source. Experiments were performed on Swiss mice and carried out in accordance to the Institute`s Bioethics Committee and IASP rules. The diabetes model was obtained by a single intraperitoneal streptozotocin (STZ; 200 mg/kg) administration. Hyperglycemic state was confirmed by measurement of blood glucose concentration and the neuropathic pain symptoms allodynia and hyperalgesia were evaluate by the von Frey and cold plate tests, respectively. The role of microglia and astroglia in diabetic neuropathy parallel with the CCL3 participation were estimated by the qRT-PCR, western blot and array protein analysis. The role of CCL3 in nociception was evaluated after its intrathecal administration. The verification of the cellular source of CCL3 were provided on the primary microglia

and astroglia culture. STZ-administration resulted in increased of plasma glucose parallel with the development of neuropathic pain symptoms 7 days after STZ-injection. Using qRT-PCR and western blot analysis the upregulation of C1q mRNA and IBA-1 protein level were observed. In contrary the downregulation of GFAP protein was detected. Furthermore, using antibody array the increase of CCL3 protein level was observed. The single intrathecal administration of CCL3 shows its pronociceptive properties in the von Frey, cold plate and tail-flick tests. *In vitro* primary cell culture studies have shown that CCL3 is of microglia and astrocytes origin. mRNA levels for CCL3 were upregulated 24h after LPS-treatment in microglia and astroglia. Our findings indicate that activated microglial and astroglial cells are response for CCL3 release and that CCL3 plays an important in nociceptive transmission which can be crucial in the development of diabetic neuropathy. Acknowledgements: Supported by 2012/05/N/NZ4/02416, 2011/03/B/NZ4/00042, KNOW scholarship and statutory funds.

T12-69B

β-amyloid plaque-associated microglia priming in transgenic mouse models of Alzheimer's disease

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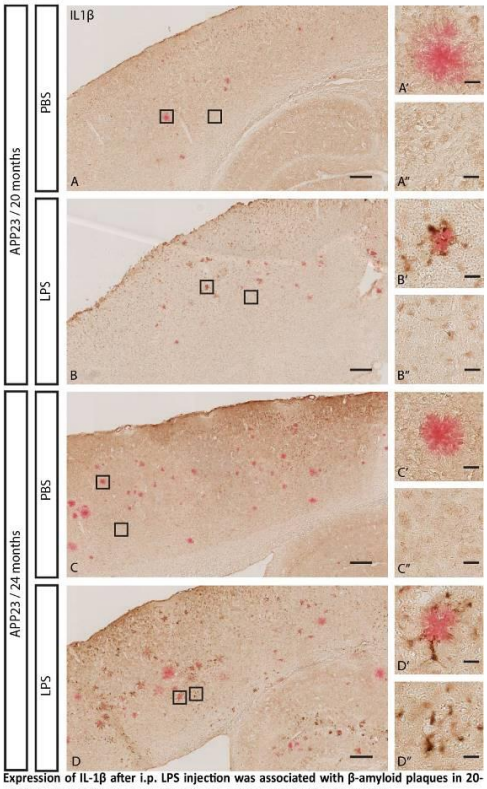
Question: The pathogenesis of Alzheimer's disease (AD), characterized by accumulation of extracellular amyloid-β (Aβ) plaques within the brain, is associated with profound microglia activation around the plaques. Furthermore, aging of the brain has been associated with enhanced sensitivity of microglia to proinflammatory stimuli, so-called microglia priming. We have investigated to which extent microglia priming is associated with Aβ plaque pathology.

Methods: For this purpose we investigated the expression of markers for microglia priming, Mac-2, CD68, MHCII, in three established mouse models for AD: APP23, APP/PS1 and 5XFAD mice.

Results: Expression of specific protein markers for microglia priming, Mac-2, MHC-II and CD68, was observed in plaque-associated microglia in 16- and 20-monthold APP23 mice, but not in microglia outside the plaque areas nor in microglia of age-matched wild type mice. The expression of Mac-2, MHCII and CD68 was enhanced after i.p. LPS injection. The expression of the pro-inflammatory cytokine interleukin-1β (IL-1β) was enhanced specifically in plaque-associated microglia in 20-month-old APP23 mice after LPS challenging. In contrast, microglia outside the plaque areas showed only moderate expression of IL-1β in response to LPS, comparable to what was observed in wild type mice. Similar, increased expression of microglia priming markers was observed in plaque-associated microglia in APP/PS1 mice and 5XFAD mice. In the cortex of old (24 months) wild type mice, specific markers for microglia priming were detected sporadically.

Conclusions: We conclude that in transgenic AD mouse models, APP23 mice, APP/PS1 mice and 5XFAD mice, microglia priming is confined to the Aβ plaque areas at early age, and later on occurs in tandem with ageing-associated priming.

Image



Expression of IL-1β after i.p. LPS injection was associated with β-amyloid plaques in 20-month-old APP23 mice, but was ubiquitous in 24-month-old APP23 mice.
Cortical sections of 20- and 24-month-old transgenic APP23 mice were stained with Congo red to label Aβ plaques and immunostained for IL-1β. In 20-month-old mice, LPS-induced IL-1β expression was confined to plaque-associated areas (B, B', B''), where no IL-1β was detected in PBS injected mice (A, A', A''). In 24-month-old mice, LPS-induced IL-1β expression was observed both associated with plaques and in non-plaque areas (D, D', D''), whereas no IL-1β expression was found in PBS-injected mice (C, C', C'').

T12-70B

Mechanisms of satellite glia-dependent spinal cord microglia activation in nerve injury-induced neuropathic pain

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Increasing evidence supports the notion that spinal cord microglia activation plays a causal role in the development of neuropathic pain after peripheral nerve injury; yet the mechanisms for microglia activation has not been elucidated. Previously, we have reported that nerve injury-induced microglia activation and subsequent pain hypersensitivity are attenuated in toll-like receptor 2 (TLR2) and Nox2 knockout mice, suggesting an important role of TLR2-Nox2-ROS signaling pathways in the nerve injury-induced spinal cord microglia activation and neuropathic pain. More recently, we found that satellite glial cell (SGC) activation and macrophages infiltration in the dorsal root ganglion (DRG) after nerve injury are also involved in neuropathic pain. To address the function SGC in the nerve injury-induced spinal cord microglia activation and pain hypersensitivity, we generated $IKK^{f/f}$/CNPase-Cre mice, in which IKK/NF-kB-dependent SGC activation is abrogated. In these mice, we found that nerve injury-induced spinal cord microglia activation and pain hypersensitivity was significantly attenuated compared to control mice. However, macrophages infiltration in the DRG *per se* has minimal effects on the spinal cord microglia activation in the nerve-injured mice. In our effort to elucidate the mechanisms of SGC activation-dependent microglia activation, we found st3gal2 is upregulated in the DRG after nerve injury, which is required for the spinal cord microglia activation. Taken together, our data demonstrate that SGC activation is responsible for the spinal cord microglia activation and suggest a putative mechanism underlying the SGC activation-induced spinal cord microglia activation and pain hypersensitivity

Poster topic 13
Neurovascular interactions

T13-01A

Extracellular vesicles from brain microvascular endothelial cell cultures promote survival, proliferation, and motility of oligodendrocyte precursor cells

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Question: We previously found that transplantation of brain microvascular endothelial cells (MVECs) greatly stimulated remyelination in the white matter lesion induced by endothelin-1 (ET-1) injection and improved the behavioral outcome (Puentes *et al.*, 2012). To help elucidate molecular and cellular mechanisms whereby MVECs stimulate remyelination of demyelinated axons, we examined the effect of extracellular vesicles (EVs) prepared from conditioned medium from MVEC cultures on oligodendrocyte precursor cells (OPCs) *in vitro*, as EVs, small membranous vesicles secreted from many types of cells, are considered to play important roles in intercellular communication (Rapaso & Stoorvogel, 2013).

Methods: MVECs were prepared from adult rat cerebra and grown in EGM-2 containing exosome-depleted fetal bovine serum (FBS). OPCs were isolated from postnatal day 1-2 rat cerebra as O4-positive cells by immunopanning and cultured in DMEM/F-12 containing platelet-derived growth factor (PDGF). EVs were obtained from conditioned medium (CM) from MVEC cultures using an exosome precipitation solution.

Results: Most cells in MVEC cultures were positive for endothelial cell markers (CD31, von Willebrand Factor (VWF)), but negative for possible contaminant cell markers, such as astrocytes (glial fibrillary acidic protein (GFAP)), smooth muscle cells (a-smooth muscle actin (SMA)), or pericytes (NG2). Most cells in OPC cultures were positive for olig2, PDGFRa, and NG2, markers for cells of oligodendrocyte lineage. Fractions obtained from MVEC-CM with the precipitation solution were positive for CD63, a marker for EVs. When they were labeled with fluorescent dye PKH67 and added to OPC cultures, they appeared to be rapidly taken up by OPCs, as we observed many fluorescent tiny vesicles within OPCs immediately after their addition. When EVs prepared from MVEC-CM were added to OPC cultures, they appeared to reduce apoptotic cell deaths of OPCs, as the number of pyknotic nuclei was significantly reduced when compared with control. They also appeared to stimulate proliferation of OPCs, as judged from increased BrdU uptake by OPCs when compared to control. Furthermore, they promoted motility of OPCs in our cell motility assay.

Conclusion: EVs derived from MVEC cultures promoted survival, proliferation, and motility of OPCs *in vitro*. Identification of molecules contained in EVs from MVECs may lead to establishment of a therapeutic strategy against ischemic demyelinating diseases.

T13-02A

Blood-brain barrier disruption: microglial responses and consequences for neural function

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Compromised blood-brain barrier (BBB) is a hallmark of neurological diseases, including Alzheimer's disease, stroke, and multiple sclerosis. However, the mechanisms regulating BBB permeability and subsequent neurodegeneration and cognitive decline remain unclear. Microglia, the resident immune cells of the CNS, continually survey blood vessel walls under normal conditions and appear to be among the first responders to both cerebral ischemia and blood leakage in the CNS. Here we investigate the role of microglia in responding to BBB opening and the consequences of this response for neural function. Using repetitive *in vivo* two-photon imaging in the cortex, we demonstrate that focal

BBB disruption in the healthy mouse brain is sufficient to induce dendritic retraction and spine elimination. Remarkably, mice genetically deficient for fibrinogen or the fibrinogen microglial receptor CD11b/CD18 are protected from these neurodegenerative changes after focal BBB disruption. In accordance, fibrinogen injection in the living mouse cortex induces dendritic retraction and spine loss in a CD11b/CD18-dependent manner and a single injection of fibrinogen in the hippocampus triggers microglial activation, neuronal loss, and impaired memory recall, as shown in the fear conditioning behavioral test. We are also currently investigating the intracellular signaling that regulates the microglia response to BBB leakage. Together, this work furthers our understanding of the cellular mechanisms underlying the brain's response to BBB disruption and has implications for developing therapeutics to treat neural injury.

T13-03A

CNS lesion-induced accumulation of platelets promotes survival of adult SVZ-derived neural stem / progenitor cells

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The presence of neural stem/progenitor cells (NSPCs) in specific areas of the central nervous system (CNS) supports tissue maintenance as well as regeneration. The subependymal zone (SEZ), located at the lateral ventricle's wall, represents a niche for NSPCs and gets activated in response to stroke or demyelination leading to migration of progenitors towards the lesion and differentiating into neurons and glia. The mechanisms that underlie this phenomenon remain largely unknown. The vascular niche and in particular blood-derived elements such as platelets, has been shown to contribute to CNS regeneration in different pathological conditions. Indeed, intracerebroventricularly administrated platelet lysate (PL) stimulates angiogenesis, neurogenesis and neuroprotection in the damaged CNS. Here, we explored the presence of platelets in the activated SEZ after a focal demyelinating lesion in the corpus callosum of mice and we studied the effects of PL on proliferating SEZ-derived NSPCs *in vitro*. We observed a lesion-induced increase in SEZ size and in the number of proliferating SEZ-resident NSPCs which correlates with the accumulation of platelets specifically along the activated SEZ vasculature. Expanding on this finding, we showed that exposure of NSPCs to PL *in vitro* led to increased numbers of cells by enhanced cell survival and reduced apoptosis without alterations in proliferation and in the differentiation potential of NSPCs. Finally, we demonstrated that the accumulation of platelets within the SEZ is spatially correlated with reduced numbers of apoptotic cells when compared to other periventricular areas. In conclusion, our results show that platelet-derived compounds specifically promote SEZ-derived NSPC's survival and suggest that platelets might contribute to the enlargement of the SEZ-NSPC pool that is available for CNS repair in response to injury.

T13-04A

Increase in astrocyte-blood vessel interaction is correlated with a decrease in the permeability of blood brain barrier during postnatal development in the cerebral cortex of Wistar rats

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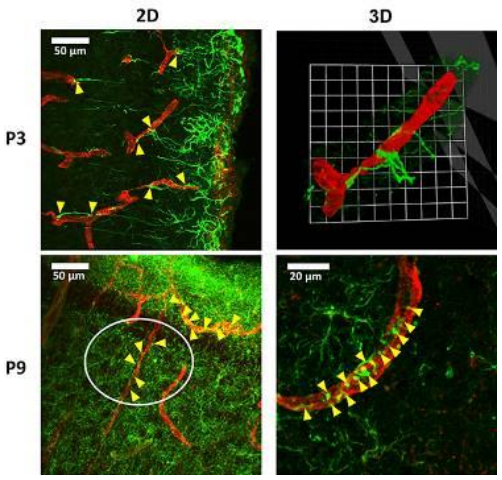
Neurovascular coupling reflects the functional metabolic and signaling relationship between the blood vasculature and neural tissue. Astrocytes are good candidates to modulate the development of neurovascular coupling since they interact with synapses and blood vessels through numerous cellular processes; however, there is a paucity of data on the development of astrocyte and vascular functional properties in the developing brain in vivo. This study tests the hypothesis that a developmental increase in astrocyte-blood vessel contact is correlated with changes in the permeability properties of blood vessels in the cerebral cortex of rat pups. To measure astrocyte-blood vessel interactions, a combination of IB4 histochemistry, astrocyte marker immunohistochemistry and confocal imaging was used to label blood vessels and astrocytes. To measure the permeability of blood vessels, a fluorescent solute TRITC-dextran (155 kD) was injected via the carotid artery and the permeability change in individual capillaries or post-capillary venules was quantified in vivo using a two-photon microscope. Our preliminary results show an increase in the area of contact between astrocytes and blood vessels (Figure1 and table 1), and a decrease in microvessel permeability from $2.9 \pm 1.1 \times 10^{-7}$ cm/s (n=11) in one-week old rats to $1.6 \pm 0.6 \times 10^{-7}$ cm/s (n=5) in two-week old rats. These results suggest that the first week of postnatal life could be important for coordinated changes in astrocytes and functional properties of the vasculature that might affect the establishment of neurovascular coupling in neonate rats. Future directions of this work include using transgenic mice expressing fluorescent reporters to obtain simultaneous measurements of astrocyte-blood vessel interactions and permeability measurements in vivo.

Image



Table

P3				P9			
Region	Vessel volume (μm^3)	Intersection volume	%	Region	Vessel volume (μm^3)	Intersection volume	%
1	1389,04	77,23	5,56	1	2598,89	1264,65	48,66
3	1137,18	58,97	5,19	2	1919,02	1153,16	60,09
4	1591,82	123,27	7,74	3	2204,36	1148,59	52,11
5	1201,82	129,23	10,75	6	1748,54	725,72	41,50
6	1917,89	52,07	2,71	7	1910,29	601,60	31,49
7	1266,81	52,45	4,14				
8	1073,35	115,16	10,73				
Average	1368,27	86,91	6,69		2076,22	978,74	46,77

***p*- value** 2,80E-06

T13-05A

Microglia have roles in both of maturation and break down of the barrier function of blood brain barrier

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The blood-brain barrier (BBB) restricts the transport of substances between vasculature and brain. It is not known whether microglia have roles in regulation of barrier function of BBB. In this study we investigated the relationship between microglia and BBB barrier function using in vitro BBB model. We first investigated the role of microglia in the maturation of barrier function of BBB. Co-existence of non-stimulated microglia with astrocytes in the brain side of the model during the maturation period significantly increased the trans-endothelial electrical resistance (TEER) and the expression levels of tight junction proteins. On the contrary, co-existence of LPS-stimulated microglia significantly decreased the TEER and the expression levels of tight junction proteins. These results suggest that microglia accelerate the maturation of barrier function of BBB but their effects are reversed in the pathological conditions. We also discovered that BBB actively transport extracellular L-Glu out from the brain. Addition of non-stimulated microglia significantly increased this transport activity via increasing the expression levels of GLAST and GLT1 in endothelial cells. However, addition of LPS-stimulated microglia reversed the effects. These results strongly suggest that microglia is important for both of maturation and break down of the barrier function of blood brain barrier.

T13-01B

Rapid tonicity induced re-localisation of endogenous aquaporin 4 in primary rat astrocytes - a therapeutic target for cytotoxic brain oedema?

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It is estimated that 69-75 million people worldwide will suffer a traumatic brain injury (TBI) or stroke each year. Brain oedema caused by TBI or following a stroke, together with other disorders of the brain cost Europe €770 billion in 2014.

Aquaporins (AQP) are transmembrane water channels involved in many physiologies and are responsible for the maintenance of water homeostasis. They react rapidly to changes in osmolarity by transporting water through their highly selective central pore to maintain tonicity and aid in cell volume regulation. We have previously shown that recombinant AQP1-GFP trafficking occurs in a protein-kinase C-microtubule dependant manner in HEK-293 cells in response to hypotonicity. This trafficking mechanism is also reliant on the presence of calcium and its messenger-binding protein calmodulin and results in increased cell surface expression of AQP1 in a time-scale of ~30 seconds. There is currently very little research into the trafficking mechanisms of endogenous AQPs in primary cells.

AQP4 is the most abundantly expressed AQP within the brain, it is localised to the astrocytic end-feet, in contact with the blood vessels at the blood-brain-barrier. In situations where the exquisitely-tuned osmotic balance is disturbed, high water permeability can become detrimental. AQP4-mediated water influx causes rapid brain swelling, resulting in death or long term brain damage. Previous research has shown that AQP4 knock-out mice were protected from the formation of cytotoxic brain oedema in a stroke model, highlighting AQP4 as a key drug target for this pathology. As there are currently no treatments available to restrict the flow of water through AQP4 as all known inhibitors are either cytotoxic or non-specific, controlling the mechanisms involved in the regulation of AQP4 in the brain could provide a therapeutic solution to such diseases.

Using cell surface biontinylation of endogenous AQP4 in primary rat astrocytes followed by neutra-avidin based ELISA we have shown that AQP4 cell surface localisation increases by 2.7 fold after 5 minutes hypotonic treatment at around 85 mOsm/kg H_2O. We have also shown that this rapid re-localisation of AQP4 is regulated by PKA, calmodulin, extra-cellular calcium and actin.

In summary we have shown that rapid translocation of endogenous AQP4 occurs in primary rat astrocytes in response to hypotonic stimuli; this mechanism is PKA, calcium, actin and calmodulin dependant. AQP4 has the potential to provide a treatment for the development of brain oedema.

Image

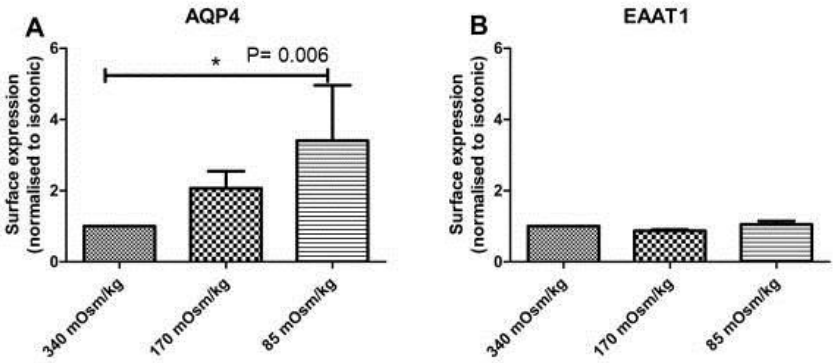

Figure 1. A) *Cell surface biotinylation of primary rat astrocytes subjected to hypotonic stress and analysis of endogenous AQP4 surface expression, n=3. B) As a negative control for translocation, membrane expression of the glutamate transporter EAAT1 was measured under the same hypotonic conditions, n=3. All data are presented as mean±SEM.* p *values and significance on the graph refer to AQP4. None of the variability in EAAT1 surface expression was statistically significant,* p = 0.37.

T13-02B

Antidepressants increase expression of the trophic factor GDF15 in astrocytes and enhance their plasticity at the glia-vasculature interface

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Morphological changes affecting the functionality of both neurons and astrocytes correlate with the onset of several neuropsychiatric disorders. Major depressive disorder (MDD) is characterized by deficits in both cell types. Recently, it has been shown that in post-mortem brains of MDD patients the coverage of blood vessels (BVs) by astrocyte processes is reduced. Antidepressant drugs (ADs) target astrocytes and regulate the expression of trophic factors which might influence those processes and reverse the disease phenotype. Our findings indicate that ADs induce an increased expression of GDF15, a neurotrophic factor, in rat primary cortical astrocytes *in vitro* and around BVs of the adult rat prefrontal cortex (PFC) *in vivo*, with concomitant formation of "bridge-like" structures between BVs and the surrounding parenchyma, possibly corresponding to astrocyte processes. The further analysis of the morphology of those processes in cultured astrocytes revealed that the AD fluoxetine (Flx) increases GDF15 expression and the numbers and lengths of those processes in wildtype cells and rescued their reduced complexity in cells derived from an animal model of depression, the high anxiety bred (HAB) rats. These results were confirmed *in vivo* in the PFC of HAB rats where we revealed a reduced coverage of BVs measured with GFAP-positive filaments, which could be reversed by Flx treatment. Our data suggest that ADs may restore the lack of blood vessels' coverage with astrocyte processes, thereby rescuing a functional glia-vasculature interface necessary to recover a physiological exchange of substances between bloodstream and brain parenchyma.

T13-03B

Volume dynamics of astroglial endfeet during cortical spreading depression

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Cortical spreading depression (CSD), a slowly propagating wave of grey matter depolarization followed by neuronal inhibition, is thought to underlie the migraine aura and similar waves occur in

cerebral ischemia, traumatic brain injury, and hemorrhage. During CSD arterioles contract despite enhanced parenchymal demand for oxygen and glucose. The mechanisms underlying neurovascular uncoupling in CSD, thought to enhance tissue damage in injuries, are unknown. The possibility that endfeet swell during CSD and modify the vascular responses has not been properly investigated. Here we use two-photon microscopy to measure the cross-sectional area of astrocytic endfeet (expressing enhanced green fluorescent protein) and neighboring arterioles (outlined with Texas Red) during CSD in isoflurane-anesthetized mice. CSD was evoked by focal application of KCl to the brain surface. CSD resulted in multiphasic changes in arteriolar cross-sectional area. The initial arteriolar contraction (~50% reduction of cross-sectional area) was associated with a profound increase in endfoot cross-sectional area. Preliminary analysis revealed that the astrocytic endfoot area almost doubled during maximal arteriolar contraction compared to pre-CSD values. Our data suggest that astrocytic endfeet swell during the early phase of CSD. The possibility that swelling-induced release of gliotransmitter contribute to neurovascular uncoupling in CSD should be explored.

T13-04B

Activity-dependent dendritic release of neuropeptides regulates neurovascular coupling in the hypothalamic supraoptic nucleus.ΔΔ

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The classical model of neurovascular coupling (NVC) implies that activity-dependent axonal glutamate release at synapses evokes the production and release of vasoactive signals from both neurons and astrocytes, which dilate arterioles, increasing in turn cerebral blood flow (CBF) to areas with increased metabolic needs. However, whether this model is applicable to brain areas that use less conventional neurotransmitters, such as neuropeptides, is currently unknown. To this end, we studied NVC in the rat hypothalamic magnocellular neurosecretory system (MNS) of the supraoptic nucleus (SON), in which dendritic release of neuropeptides, including vasopressin (VP), constitutes a key signaling modality influencing neuronal and network activity. Using a multidisciplinary approach, we investigated VP-mediated vascular responses in SON arterioles of hypothalamic brain slices of Wistar or VP-eGFP Wistar transgenic rats. Bath-applied VP significantly constricted SON arterioles (Δ-41±7%) via activation of the V1a receptor subtype. Vasoconstrictions were also observed in response to single VP neuronal stimulation (Δ-18±2%), an effect prevented by V1a receptor blockade (V2255), supporting local dendritic VP release as the key signal mediating activity-dependent vasoconstrictions. Inhibition of glia function with the gliotoxin L-AAA (2 mM) did not compromise VP mediated vasoconstriction. However, L-AAA significantly reduced basline vascular tone (Δ14±5%) Differently from single cell stimulation, osmotically-driven magnocellular neurosecretory neuronal population activity resulted in a predominant nitric oxide (NO)-mediated vasodilation (Δ19±2%). Activity-dependent vasodilations were followed by a VP-mediated vasoconstriction, which acted to limit the magnitude of the vasodilation and served to reset vascular tone following activity-dependent vasodilation. Taken together, our results unveiled a unique and complex form of NVC in the MNS, supporting a competitive balance between NO and activity-dependent dendritic release of VP, in the generation of proper NVC responses. Moreover, our results indicate that while astrocytes in the SON may contribute to the modulation of steady-state vascular tone, they are not key intermediaries in VP-mediated vasoconstriction of SON arterioles, or the NVC response to hyperosmotic stimulation.

T13-05B

Aquaporin 4 is involved in brain edema and blood-brain barrier disruption induced by methamphetamine

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Methamphetamine (METH) is a highly addictive psychostimulant which consumption in Europe has been increased over the last years. Despite the well described neurotoxicity of METH, many questions

remain unanswered. More recently, some studies have suggested that this drug can also compromise the blood-brain barrier (BBB) properties. Additionally, it is known that METH use may cause cerebral edema, and disturbance in the well-regulated water homeostasis will lead to brain edema that can culminate in brain dysfunction. Importantly, water transport at BBB is regulated by water channels, the aquaporins (AQPs), with AQP4 being the most important at the Central Nervous System. In fact, AQP4 is express in astrocytic end-feet that surround brain endothelial cells. Furthermore, AQP4 has two isoforms, M1 and M23, and the ratio M23/M1 regulates the brain water homeostasis since M23 stabilizes the channel function, whereas M1 disrupts the AQP4 structure. Indeed, brain edema has been observed in several neuropathologies, including under conditions of METH consumption, but nothing is known about the underlying mechanisms.

The present work aims to investigate the role played by AQP4 in METH-induced BBB dysfunction and edema formation. Our results show that METH (4× 10 mg/kg, 2h apart) increased water content in both mice striatum and hippocampus, together with a clear BBB breakdown and microvessels weakening. In order to clarify the role of AQP4 on these alterations, we further demonstrated that the blockade of AQP4 with a specific inhibitor (TGN-020, 200 mg/kg 30 min before the first METH injection) attenuated METH-induced edema and BBB disruption. Moreover, METH interfered with AQP4 expression in the abovementioned brain regions causing an increase in the M1/M23 ratio, which is an indicator of water homeostasis disruption. Furthermore, we demonstrated that brain edema and BBB permeability triggered by METH also involved the protein kinase C (PKC) pathway since its inhibition with chelerythrine (4x 5 mg/kg, 1h before each METH injection) prevented both effects.

Overall, our results demonstrate that METH interferes with AQP4 expression leading to both brain edema and BBB dysfunction *via* PKC signaling pathway.

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T13-06B

Mechanosensitive Piezo2 channels are functionally expressed in retinal astrocytes: implications for blood flow autoregulation

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Question: Retinal astrocytes are known to play a critical role in the development of the retinal vasculature, in regulating retinal homeostasis and in maintaining the inner blood retinal barrier. They have also been implicated in retinal blood flow control, but the mechanisms through which they modulate vascular tone are poorly understood. Here we show that astrocytes express the novel mechanosensitive ion channel, Piezo2, which we believe may allow them to monitor and modulate the contractile state of the retinal vasculature.

Methods: Piezo2 expression was investigated by immunolabelling of rat retinal flatmount preparations and human retinal astrocytes in culture. siRNA knockdown and Fura-2 calcium microfluorimetry in human retinal astrocytes was used to investigate functional Piezo2 channel activity.

Results: Piezo2 was expressed in retinal astrocytes. Piezo2 expressing end foot projections were observed to wrap around the vasculature and connexion 43 (GJP1) was observed to be expressed in the regions between the end foot processes and the underlying smooth muscle layer of retinal arterioles. Cultured human retinal astrocytes also expressed Piezo2. Hypotonic-induced stretch of retinal astrocytes evoked a transient increase in intracellular calcium which was maintained in the presence of the swell-activated chloride channel inhibitor 1mM DIDS. This response was significantly inhibited in cells transfected with siRNA against Piezo2, but not scrambled siRNA, strongly suggesting that this stretch-activated response is produced by the action of the Piezo2 channel.

Conclusion: Piezo 2 channels are expressed at a molecular and functional level in retinal astrocytes. Their potential activation in astrocytic end-feet during periods of increased blood pressure and their precise role in modulating retinal vascular tone now warrants further investigation.

Poster topic 14
Regeneration and repair

T14-01A

Functions of histone deacetylases in Schwann cells during regeneration

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Axons are surrounded by myelin sheaths, which are formed by glial cells called Schwann cells in the peripheral nervous system (PNS) and oligodendrocytes in the central nervous system (CNS). These myelinating glial cells have two main functions, providing axonal insulation and saltatory nerve conduction. In addition, Schwann cells play a key role during the regeneration of the PNS. Indeed, they can efficiently promote axonal regrowth, due to their capacity to de-differentiate and re-differentiate after a lesion, whereas oligodendrocytes in the CNS cannot.

In search of a master control of Schwann cell plasticity, we decided to investigate the functions of chromatin remodeling enzymes. Acetylation and deacetylation of histones on lysine residues are key modifications for chromatin remodeling. Histone deacetylases (HDACs) control gene expression by remodeling chromatin and modifying the activity of transcription factors. There are 18 known mammalian HDACs, subdivided into four classes, based on their structure. Using mouse genetics, we have previously shown that the two highly homologous class I HDACs, HDAC1 and HDAC2 are crucial for survival and myelination of Schwann cells, and our data indicate that these two HDACs are also required for the maintenance of peripheral nerve integrity in adults. Our current aim is to understand whether and how HDACs can influence the regeneration process in Schwann cells.

We found that HDAC1 and HDAC2 were strongly upregulated during regeneration after a sciatic nerve crush lesion in adult mice, suggesting important functions in this process. To analyze the potential functions of HDAC1 and HDAC2 in Schwann cells during regeneration, we generated a tamoxifen-inducible conditional knockout mouse line where we ablated HDAC1 and HDAC2 in adult mice specifically in Schwann cells. In the absence of HDAC1 and HDAC2, Schwann cells de-differentiated faster after lesion, and remyelination was thinner. We found that the transcription factors Pax3, Oct6 and Krox20 and the major component of the myelin sheath myelin protein zero (P0) were strongly reduced 1 day, 3 days, 12 days and 1 month post crush lesion, respectively.

We are currently investigating the molecular mechanisms responsible for these functions.

T14-02A

Applying mechanistic models to prove that an M1-to-M2 polarization switch in microglia and macrophages can happen at the initiation of remyelination in cuprizone-induced demyelinating lesions

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In the context of acute demyelination induced by stereotaxic injection of lysolecithin, Miron et al [1] have observed an M1-to-M2 polarization switch at the initiation of remyelination in both microglia and peripherally-derived macrophages, corresponding to the time of differentiation of oligodendrocytes. Let *h* be the following hypothesis: “An M1-to-M2 polarization switch happens at the initiation of remyelination”. The authors report that *h* has not been observed in experiments that employ acute and chronic cuprizone models of induced demyelination (see, for example, Armstrong et al [2]). Miron et al [1] point out that this likely reflects the confounding effects of overlapping de- and remyelination phases in these models.

In oncology research, Gatenby and Maini [3] encourage the use of mathematical, mechanistic models, which, informed by extant data and continuously revised by new information, guide further experimental design and interpretation.

We have translated Miron's and Armstrong's experiments into two mathematical sets with a number of relations and constraints; we call these sets "models" because they are representations of reality [4]. Next, we have translated *h* into a logical predicate, and we have employed an automatic inference engine (alloy.mit.edu) to check whether the predicate is logically satisfied by the models or not. As expected, Alloy's output confirms that *h* is logically true in the model representing Miron's experiment. Interestingly, when asked about the model representing Armstrong's experiment, Alloy concludes that the hypothesis is logically true in some instantiations of the model. To confirm this, the tool generates a number of instances where *h* holds: each one represents a possible replica of Armstrong's experiment. Thus, in one of the generated instances, the M1-to-M2 switch happens at the initiation of remyelination in the third week of cuprizone-based diet, while in another instance (Fig. 1) the switch and the initiation of remyelination happen after five weeks of cuprizone ingestion. While these results are predictions generated by a computer, they prove that remyelination during cuprizone-induced demyelination is logically consistent with hypothesis *h*, and they illustrate how mechanistic models can be applied to neurology as a useful complement to empirical research, as it happens in most scientific disciplines.

For Ann and Bart.

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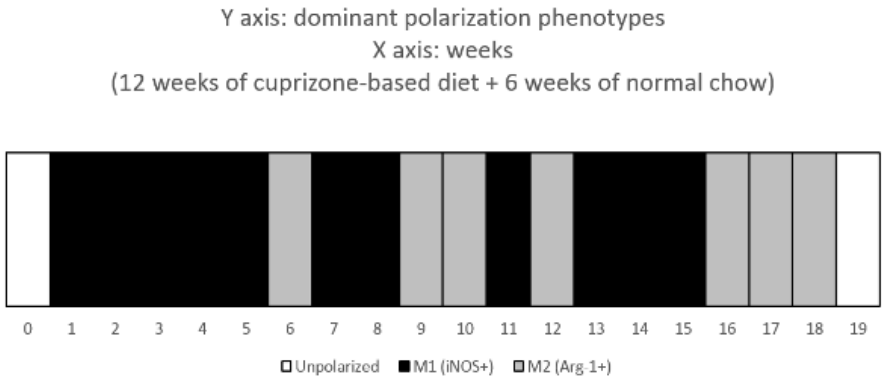
Image



T14-03A

Region and dynamic specificities of adult neural stem cells and oligodendrocyte precursors in myelin regeneration in the mouse brain

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Myelin regeneration can occur in the brain following demyelination. Parenchymal oligodendrocyte progenitors (pOPC) are known to play a crucial role in this process. Neural stem cells residing in the

ventricular-subventricular zone (V-SVZ) also have the ability to generate oligodendrocytes but their contribution to endogenous myelin repair was so far considered to be negligible. Here, we addressed the relative contribution of pOPC and VSVZ- derived neural progenitors (SVZdNP) to remyelination in cuprizone mouse models of acute or chronic corpus callosum (CC) demyelination. Using genetic tracing, we uncover an unexpected massive and precocious recruitment of SVZdNP in the anterior CC after acute demyelination. These cells very quickly adopt an oligodendrocytic fate and robustly generate myelinating cells as efficiently as pOPC do. In more posterior areas of the CC, SVZdNP recruitment is also observed but less important whereas pOPC are more efficiently activated, underlining a regionalization in the mobilization of these two cell populations. Strikingly, in a chronic model when demyelination insult is sustained in time, SVZdNP minimally contribute to myelin repair, a failure associated with a drastic drop of SVZ cell proliferation. In this context, pOPC remain reactive even though less efficiently than in the acute model, and become the main contributors to myelin regeneration. Altogether our results highlight a region and context-dependent contribution of SVZdNP to myelin repair that can equal pOPC. They also raise the question of a possible exhaustion of SVZ proliferation potential in chronic pathologies.

T14-04A

Non-steroidal anti-inflammatory drug indometacin enhances endogenous remyelination

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Multiple sclerosis is the most frequent demyelinating disease in the CNS that is characterized by inflammatory demyelinating lesions and axonal loss, the morphological correlate of permanent clinical disability. Remyelination does occur, but is limited especially in chronic disease stages. Despite effective immunomodulatory therapies that reduce the number of relapses the progressive disease phase cannot be prevented. Therefore, promotion of neuroprotective and repair mechanisms, such as remyelination, represents an attractive additional treatment strategy. A number of pathways have been identified that may contribute to impaired remyelination in MS lesions, among them the Wnt/ß-catenin pathway. The aim of the presented study was to determine whether well-known drugs that modulate the Wnt/ß-catenin pathway promote oligodendroglial differentiation and myelination. The non-steroidal anti-inflammatory drug indometacin promotes differentiation of primary human and murine oligodendrocytes, myelination of cerebellar slice cultures and remyelination in cuprizone induced demyelination. Our *in vitro* experiments using GSK3ß inhibitors, luciferase reporter assays and oligodendrocytes expressing a mutant, dominant stable ß-catenin indicate that the mechanism of action of indometacin depends on GSK3ß activity and ß-catenin phosphorylation. In summary, we demonstrate that indometacin, a drug with a well-known safety-profile, enhances oligodendroglial differentiation and remyelination via modulation of the Wnt/ß-catenin pathway and therefore might represent a promising treatment option to enhance endogenous remyelination in MS patients.

T14-05A

Intraventricular injection of mesenchymal stem cells in a chronic demyelinated murine model, promotes functional recovery by stimulating the endogenous oligodendrogenic program

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Both in our lab as well as others have shown the therapeutic potential of mesenchymal stem cells (MSCs), including demyelinating diseases. The aim of this work is to investigate their potential in a chronically demyelinated mouse model. To this end, bone marrow-derived MSC were pre-incubated *in vitro* with iron nanoparticles and stereotaxically injected into both lateral ventricles of mice fed with

cuprizone for 12 weeks, inducing an irreversible demyelinating state. After transplantation, all the animals were analyzed *in vivo* by MRI at different time points (0-90 days). The MRI images were processed to quantify myelin in the corpus callosum using image analysis software. Also, several mice were sacrificed at the same time points to perform immunohistochemistry analysis for several immature and mature oligodendrocyte markers. Increased myelin content as well as the number of cells expressing either immature or mature oligodendrocyte markers was detected in the corpus callosum as early as two months after MSC transplantation, in the stem cell-treated mice. To corroborate the functional consequences of this myelin increase, the axonal conduction velocity in the CC was analyzed by electrophysiological recordings, which showed a functional recovery over time in the MSC-treated group. Similarly, three months after transplantation, the MSC were still present within the cerebrospinal fluid of the ventricles, where it was also found an overexpression of several trophic factors such as PDGF, IGF, NT3, NT4 and FGF2, which are likely to be candidates for the regenerative effect previously observed. Moreover, preliminary data obtained with BrdU incorporating assay, suggest that this recovery could be due to a paracrine effect of the MSCs in the subventricular zone, where they would be stimulating the endogenous neurogenic process and oligodendrogenic fate decision on the neural progenitor stem cells. Altogether, these findings revealed that MSC transplantation may have major implications for the development of future therapeutic strategies for chronic demyelinating disorders.

T14-06A

Tissue plasminogen activator (tPA) acts on oligodendrocytes and promotes remyelination after white matter damage

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Multiple sclerosis (MS) is a chronic disease of the central nervous system (CNS) characterized by successive cycles of demyelination and remyelination. Several studies suggest that tissue plasminogen activator (tPA), a serine protease expressed in the CNS, could be involved in MS.We report that the outcome of experimental autoimmune encephalomyelitis (a classical model of MS) is worse in tPA knockout mice than in wild type mice. We hypothesized that this result could be due to an effect of tPA on demyelination and/or remyelination.

To test this, we used a model of focal demyelination (stereotactic lesion of the corpus callosum), in which we compared over time the evolution of lesions between tPA knockout mice and wild type mice.

We observed by T2-weighted 7T-MRI that tPA knockout mice displayed bigger white matter lesions and a slower recovery. Moreover, histological analysis showed a reduced number of oligodendrocytes within the lesions of tPA knockout mice. Finally, we addressed the issue of the cellular origin of tPA during white matter regeneration and alongside performed *in vitro* assays to further investigate if these results were due to an effect of tPA on oligodendrocyte maturation, proliferation and/or migration.

In conclusion, this study suggests positive actions of tPA on remyelination, suggesting potential benefit in white matter injury occurring in CNS disease such as MS.

T14-07A

Spatio-temporal proteins study of rat spinal cord injury and glial cells involvement

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Proteomic analysis of molecules released by spinal cord (conditioned media CM) from different segments: lesion site and all the others segments rostral and caudal at different time after injury 3, 7

and 10 days was performed in order to describe the molecular environment. Inflammation at the acute phase is very important for the injury, in first step to promote macrophage activation to clean debris and damaged cells, to allow astrocytes to protect healthy cell with glial scare formation but if inflammation persists to chronic phase, the process become deleterious.

To better understand the role of immune response in secondary damage processes, a balloon-compressive technique was used to produce spinal cord injury at thoracic Th8-9 spinal level in adult rat. Proteins CM were analyzed by shot-gun analyses. Immunohistochemistry were performed on interested segment C1 to understand what is happen in cellular level. Changes in microglia M1/M2 polarization were studied through CX3CR1, CD206 and CD86 expression.

Proteomics data shown interesting clusters depending time or segments. Controls are identified to be completely different from injured CM. Lesion from 3, 7, and 10 days are grouped together and completely distinct of other segments.

Results showed some specific proteins at each site of the lesion. Among proteins from rostral and lesion segments, some are related to chemokines or to neurogenesis factors. In contrast, proteins from caudal segments are more related to necrosis factors. Neurotrophic factors have been highly identified at 3 days, diminished at 7 days and disappeared at 10 days e.g (CTGF, NOV, FGF-1, NGF, TGF beta (1-3), GAP-43, neurotrimin). However at 10 days, proteins related to synaptogenesis have been detected e.g. R-SNARE synaptobrevine, Q-SNAREs (SNAP25), GTPases (Rab proteins family), syntaxines, synaptotagmine. These data showed that at 10 days proteins involved in axonal reconnection and synaptic transmission reflecting that a neurorepair process has started. In contrast, at the caudal segment, the protein profile is always inflammatory and apoptotic whatever the days after SCI. Microglia cells Iba1-positive are activated after SCI in both rostral and caudal segment. CD206 and CX3CR1 marker for M2 anti-inflammatory phenotype are both increased expression in rostral segment with over-expression for CX3CR1 in rostral compared to caudal.

Taken together, these data shown that polarization in terms of inflammatory and neurotrophic responses occurs between rostral and caudal segments in SCI between 3 to 10 days post-lesion

T14-08A

Control of oligodendrocyte plasticity by histone demethylases after spinal cord injury

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Chromatin remodeling is a key process that controls gene expression during development. We hypothesize that chromatin remodeling is critically involved in the plasticity of neural cells after a nerve injury. With this research proposal, our aim is to understand the involvement of chromatin remodeling enzymes in the regeneration of the nervous system and to use this newly acquired knowledge to increase the plasticity of the nervous system and improve regeneration after a spinal cord injury.

Local changes of chromatin architecture can be achieved by post-translational modifications of histones such as methylation, acetylation, phosphorylation, ubiquitination, sumoylation, and ADP-ribosylation. These changes are dynamic and allow for rapid repression or de-repression of target genes. We [1] and other groups have shown the key roles of chromatin remodeling enzymes in the development of myelinating cells (reviewed in [2, 3]). Comparatively, the role of chromatin remodeling in myelinating glia during regeneration is mostly unknown.

My project tackles and compares the essential functions of histone demethylation in Schwann cells and oligodendrocytes during regeneration after a nerve lesion.

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T14-09A

Role of heparan sulfate in the control of myelin regeneration

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Myelin regeneration can occur in the brain following demyelination. Both Parenchymal oligodendrocyte precursors (OPC) and Subventricular zone derived neural progenitors can repopulate the lesion and replace lost oligodendrocytes in mouse brain. In the aim to identify new potential factors involved in this remyelination process, a microarray analysis was performed to compare gene expression in purified OPCs from healthy and demyelinated mouse brain. Among genes showing a specific modification in OPCs after demyelination, we identified an up-regulation of *Ndst1*, a key enzyme of the heparan sulphates (HS) synthesis. HS are known to modulate the activity of morphogens and trophic factors involved in cell proliferation, migration and differentiation processes. We analyzed the impact of the heparan sulphates polysaccharide chains hydrolysis on purified OPCs behavior in culture and showed that heparan sulphates promote OPCs migration and prevent oligodendrocytes maturation. Using an acute model of focal demyelination of the corpus callosum, we show that *Ndst1* and heparan sulphates form a belt around the demyelination region and are expressed by both OPCs and oligodendrocytes during the demyelination and remyelination phases. The functional involvement of *HS* in the repair process is evaluated by analyzing the demyelination and remyelination processes in a mouse model allowing conditional deletion of ndst1 in oligodendrocytes. Our present data on the *Ndst1* function suggest a role for HS on the establishment of a reactive microenvironment around the lesion instructive for the mobilization process and permissive for myelin repair.

T14-10A

Juvenile ependymal cells show greater self-renewal potential and generate more oligodendrocytes than adult cells after spinal cord injury

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Traumatic spinal cord injury (SCI) is a pathological condition that can occur at any age, but statistics show a higher incidence in young adults. SCI typically leads to paralysis and is currently incurable.

Importantly, the damaged spinal cord shows little regenerative ability, due to limited axonal regrowth and the formation of an inhibitory glial scar around the lesion site. Recent findings show that ependymal cells, the endogenous spinal cord stem cells, and their progeny respond to injury contributing to cell maintenance, reducing tissue damage, and releasing pro-axonal growth molecules. However, our understanding of the regenerative and self-repair mechanisms involving this cell population after SCI is still largely unknown.

Here, we investigated the effect of ageing on the stem cell potential and injury-induced activation of the ependymal cells *in vitro* and *in vivo*, taking advantage of FoxJ1-CreERT2:YFP transgenic mouse line. We showed that self-renewal capacity of ependymal cells declines with ageing *in vitro*. However, the self-renewal potential of neurospheres derived from juvenile mice (P21) is rescued back to perinatal levels after SCI. Importantly, neurospheres derived by injured juvenile mice generate more oligodendrocytes compared to those derived from adult mice, suggesting a negative effect of ageing on the remyelination of regrowing axons. We also demonstrated that juvenile ependymal cells are activated and recruited to the injury site after SCI *in vivo* and we are currently investigating the effect of ageing on the contribution of the activated ependymal cells to scar formation and mechanisms of self-repair.

In summary, we showed that juvenile ependymal cells have an intrinsic higher stem cell potential compared to adult ependymal cells. Altogether, this suggests that the modulation of the ependymal

cells response to SCI can represent a therapeutic strategy to treat paralysis especially effective in young patients.

T14-11A

Role of L-PGDS in PNS regeneration and remyelination

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Axonal NRG1 type III is an essential instructive signal for PNS myelination, since its expression determines whether axons are myelinated and the thickness of the myelin sheath. NRG1 type III, similar to other growth factors, follows a classical intramembrane proteolysis processing mediated by the γ-secretase complex. NRG1 type III γ-secretase cleavage generates an intracellular fragment that translocates in the nucleus to upregulate the expression of the Prostaglandin D2 Synthase (L-PGDS) gene in neurons, an enzyme that catalyzes the conversion of prostaglandin H2 into prostaglandin D2 (PGD2). Specific inhibition of L-PGDS activity impairs *in vitro* myelination. Accordingly, myelin in L-PGDS null mice is noticeably thinner, presenting L-PGDS as a novel modulator of PNS myelination.

Notably, arachidonic acid and its metabolites prostaglandins are involved in Wallerian Degeneration and axonal regeneration after injury. Thus, investigating whether L-PGDS signaling is maintained after injury could be extremely important to favour nerve regeneration and remyelination after an injury event.

Accordingly, in preliminary experiments we found that L-PGDS is upregulated after crush injury. Next, to understand whether L-PGDS plays a role in remyelination we performed sciatic nerve crush injury in 2 months old L-PGDS null and WT control animals and we analysed crushed nerves by morphologic and biochemical approaches at three different time points (T7 - T21 - T60). Preliminary results suggest that L-PGDS might be dispensable for remyelination, however more thorough analyses need to be performed.

T14-12A

Vulnerability and fate of oligodendroglia in areas of secondary degeneration following neurotrauma

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Following a traumatic insult to the central nervous system (CNS), a cascade of cellular and molecular processes culminate in the spread of the injury from the inceptive site to surrounding, uninjured tissue. This 'secondary degeneration' is characterised by myelin decompaction and loss. Endogenous mechanisms are initiated to replace and/or repair myelin, including the proliferation and maturation of oligodendrocyte progenitor cells (OPCs) into myelinating oligodendrocytes. However, the persistence of decompacted myelin suggests that OPC maturation is dysfunctional following injury. Additionally, our previous findings have suggested that OPC numbers are chronically depleted in tissue vulnerable to secondary degeneration. Oxidative stress is a characteristic feature of secondary degeneration which may be associated with OPC death and interfere with the differentiation of OPCs into myelinating oligodendrocytes; however, evidence is lacking in support of this hypothesis.

The aim of our study is therefore to ascertain whether OPCs vulnerable to secondary degeneration undergo oxidative damage, and relate this to their proliferation, maturation into myelinating oligodendrocytes and/or death.

Methods: A 200um partial transection was administered to the dorsal aspect of the optic nerve in adult female PVG rats, and the ventral optic nerve vulnerable to secondary degeneration was assessed.

Intraperitoneal injection of Ethynyl deoxyuridine (EdU) within the 3 days following injury was used to map the fate of proliferating OPCs. The vulnerability of OPCs, newly differentiated and pre-existing myelinating oligodendrocytes to oxidative stress was assessed immunohistochemically and TUNEL used for quantification of cell death.

Results: The number of proliferating OPCs increased at days 3 and 7 following injury compared to controls ($P \leq 0.05$). Despite this, there was a decrease in the total number of OPCs at day 7 following injury but no significant difference in myelinating oligodendrocyte numbers at days 3 and 7 following injury, compared to controls. Preliminary findings indicate an associated increase in lipid peroxidation in OPCs ($P \leq 0.05$), regardless of proliferative state, which was not observed in myelinating oligodendrocytes.

Conclusions: These results indicate that OPCs have a vulnerability to lipid peroxidation that is not observed in mature oligodendrocytes. Moreover, the numbers of mature oligodendrocytes are maintained following injury despite chronic myelin decompaction.

T14-13A

Mitochondrial regulation of astrocyte reactivity in response to inflammatory insult

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In the adult brain, astroglial cells play important roles in regulating synaptic homeostasis, neurovascular coupling and in the processes associated to pathological states. In particular, in response to injury and the ensuing inflammation astrocytes enter a state of reactivity (i.e. reactive astrogliosis) which is believed to contribute to regulation of tissue repair. While emerging evidence suggests that this state of reactivity is mirrored by unique transcriptional changes in the metabolic signature of astrocytes, how these changes are translated into subcellular modifications that allow for astrocytic response to local inflammation is poorly understood. Recently, we have shown that astrocytes possess the capability to adapt their energy metabolism via regulated remodeling of their mitochondrial network *in vivo* and in brain slices. In particular, rapid changes (i.e. within 30 minutes) in mitochondrial dynamics in astrocytes exposed to focal pro-inflammatory stimuli appeared regionalized within branches of the cell proximal to the insult. This observation prompted us to examine whether local changes in astrocyte mitochondrial networks may lead to similar compartmentalized alterations in signaling pathways essential to the astrocytic function. Namely, whether regulated changes in mitochondrial function in response to brain injury and local inflammation may contribute to scar formation and tissue repair. Methodologically, we will perform targeted manipulation of specific effectors of mitochondrial dynamics in astrocytes to assess their functional role to scar formation over time following cortical injury. This will be achieved via a combination of mouse genetics and imaging techniques to dissect the local contribution of mitochondrial function to astrocytic reactivity.

T14-14A

Analyses of epigenetic change in the injured mouse spinal cord

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Purpose: Spinal cord injury (SCI) triggers dynamic changes in the local environment including a robust immune response characterized by cytokine production, infiltration of inflammatory cells and proliferation of neural cells. These processes following initial injury may result in deterioration of motor/sensory functions due to glial scar-formation as well as neuronal death and de-myelination. Although epigenetic modification is deeply involved in development and disease-progression, cell-type specific dynamisms of epigenetic modification within the injured spinal cord are not fully analyzed so far. Here, we examined the expression of epigenetic modifiers regulating DNA methylation to reveal the change of expression of epigenetics-related genes after SCI.

Materials and Methods: Nine weeks old female C57BL6 mice were used for all the experimental groups. Mice were anesthetized, and their spinal cords were exposed by laminectomy at T10. Contusive SCI was induced using a commercially available SCI device (IH impactor, 60kdyn; Precision Systems and Instrumentation, Lexington, KY) as described previously. One or two weeks after injury, animals were anesthetized and transcardially perfused with 4% paraformaldehyde in 0.1 M PBS. Then the spinal cords were removed and sectioned for immunohistological analyses. The proliferation of progenitor cells, or expression of DNA methyltransferases (DNMTs) was analyzed by immunohistochemistry. The methylation pattern of progenitor cells was analyzed by whole genome bisulfate sequencing. Also, target genes of the elevated DNMT were analyzed by chromatin immunoprecipitation (ChIP) and following DNA sequencing.

Results: Among DNMTs, DNMT3a expression was upregulated after SCI. Interestingly, this upregulation was observed in proliferating neural cells, which were further characterized as oligodendrocytic progenitors. To further characterize and estimate biological significance of the upregulation of DNMT3a, we performed ChIP-sequencing using antibody against DNMT3a together with single nucleotide resolution-methylome analysis and found potential targets of DNMT3a.

Conclusion: The expression of DNMT3a was elevated after SCI, and its target genes were suggested to effect the change of the microenvironment after the injury. Future analysis and characterization of epigenetic events might lead to novel intervention for patient with SCI.

T14-15A

17β-estradiol augments axotomy-induced cell-type specific changes in P2X7 receptor expression in the mouse hypoglossal nucleus

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Question: We have studied whether 17β-estradiol affects P2X7 receptor expression and distribution in the axotomized mouse hypoglossal nucleus.

Methods: The left hypoglossal nerve of ovariectomized mice was cut, then animals received a single injection of 17β-estradiol (25μg/100 g b. w. in 20% 2β-hydroxy-cyclodextrin) or vehicle one hour after axotomy. Mice were sacrificed on day 4 following surgery. The immunoreactivity pattern of P2X7 receptor, estrogen receptor alpha / beta and the microglia marker CD11b was analyzed on cryostat sections using the Image J software. P2X7 receptor concentrations were measured using semiquantitative Western blots on protein samples obtained with non-contact laser capture microdissection of the two sides of the hypoglossal nucleus.

Results: Following axotomy less P2X7 immunolabeled neurons and an increase in area fraction of microglial P2X7 receptor immunolabeling were observed on the operated side compared to the intact side of the hypoglossal nucleus. In 17β-estradiol-treated mice a significant decrease in area fraction of neural and a significant increase in area fraction of microglial P2X7 immunostaining were detected on the operated side compared to animals injected with vehicle. P2X7 immunoreactivity pattern on the intact side of the nucleus was not different from that seen in control, sham operated mice, and it was not influenced by 17β-estradiol. Semi-quantitative Western blots showed a significant decrease in P2X7 protein concentration on the lesioned side compared to the intact side in animals injected with 17β-estradiol. CD11b immunoreactive microglia area fraction was not affected by 17β-estradiol, and neither estrogen receptor alpha, nor beta colocalized with CD11b.

Conclusions: Our results suggest that the axotomy-induced cell-type specific changes in P2X7 receptor expression may be augmented by 17β-estradiol. A direct cross-talk between 17β-estradiol and P2X7 signaling may take place in injured hypoglossal neurons, whereas estrogen effect on P2X7 receptor expression may not directly depend on ER action in stimulated microglia.

T14-16A

Alternatively activated brain-infiltrating macrophages facilitate recovery from intracerebral hemorrhage

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Intracerebral hemorrhage (ICH) is one of the major causes of stroke, constituting 10% of all stroke cases. Upon ICH, the primary mechanical insult by increasing volume of hematoma is followed by massive infiltration of macrophages in the perihematoma regions. Recently, it has been reported that the function of macrophages is distinct depending on their activation type; M1, or classical activation, leads to pro-inflammatory responses, while M2, or alternative activation, contributes to tissue repair or healing. Since the function of brain-infiltrating macrophages after ICH has not been elucidated, we characterized the phenotype of macrophages infiltrating brain parenchyma and investigated their role in ICH. Upon ICH injury, the number of brain-infiltrating macrophage increased in perihematomal region. To investigate the role of these brain-infiltrating macrophages, we depleted peripheral monocytes by i.p. clodronate liposome injection. In the monocytes-depleted mice, the ICH-induced neurological deficits were more severe compared to those of control mice, indicating a protective role of macrophages in ICH injury. The mRNA expression of Arginase-1 (an M2 marker) was upregulated in the ICH-injured brain, while iNOS (an M1 marker) expression was not significantly altered. In flow cytometry, mannose receptor (an M2 marker)-expressing macrophages increased at a delayed time point after ICH. The M2 polarization of the brain-infiltrating macrophages suggested that the brain microenvironment around macrophages may affect macrophage activation. To explore such possibility, bone marrow derived macrophages (BMDM) were co-cultured with mouse brain mixed glia cells (MBMG) and then tested for their activation phenotype. Upon co-culture with MBMG, the number of mannose receptor-positive BMDMs was significantly increased, which suggests that glia cells can induce macrophage M2 polarization. Furthermore, treatment with MBMG conditioned media increased the number of mannose receptor-expressing BMDM and the mRNA expression of Arg-1 and Ym1. Taken together, these data suggest that brain-infiltrating macrophages, after ICH, are differentiated to the M2 phenotype by brain glial cells, and thereby contribute to the recovery from ICH injury.

T14-17A

Cend1 and Neurogenin-2 drive neuronal reprogramming of astrocytes *in vitro* and *in vivo* following brain injury

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Reprogramming of non-neuronal CNS cell types to neurons through forced expression of neurogenic factors has been recently suggested as a promising method for *in vivo* neuronal replacement in the injured brain. More specifically, it has been shown that astrocytes, which constitute the most abundant cell type of the human brain, have the capacity to form various subtypes of neurons *in vitro*, and to some extent, *in vivo*. Here, we study the effect of forced expression of two factors known to promote neurogenesis during development, BM88/Cend1 and Neurogenin-2 (Ngn2), in instructing neuronal reprogramming of cortical astorocytes both *in vitro* and *in vivo.* In our study, overexpression of Cend1, Ngn2 or a combination of both in P5 cortical astrocytes *in vitro* using recombinant retroviral vectors, resulted in acquisition of induced neuronal cells expressing βIII-tubulin, synapsin, and the neuronal subtype-specific markers, GABA, tyrosine hydroxylase (TH), and glutamate. To investigate if the two molecules exert the same reprogramming action *in vivo*, we are currently using retro-, lenti-, and astrocyte-specific lentiviral vectors expressing Cend1 or Ngn2, that we sterotaxically inject alone or in combination in a model of penetrating, mechanical cortical injury. Our first data suggest that 20 days following injury and subsequent viral transduction, a significant percentage of Cend1 and/or Ngn2-transduced cells in the astrogliosis area surrounding the lesion site exhibit neuronal morphology, while they express the early and late neuronal markers doublecortin and NeuN, respectively. These data suggest an important role for Cend1 and Ngn2-mediated astrocytic reprogramming *in vivo*. More molecular phenotype analysis and *in vivo* imaging studies are in progress to further characterize the properties of Cend1- and Ngn2-transduced cells.

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T14-18A

Glutaredoxin 2 increases oligodendroglial capacity for regeneration after neuroinflammatory damage

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Regeneration of brain lesions induced by chronic autoimmune neuroinflammation depends on migration of oligodendroglial progenitor cells into the damaged area, survival of these cells in the toxic environment of the lesion, and re-myelination of axons. Here, we provide evidence that glutaredoxin 2, a vertebrate specific oxidoreductase of the thioredoxin family, promotes all these regenerative functions in models mimicking neuroinflammation.

Our findings revealed that increased amounts of glutaredoxin 2 lead to an increased amount of differentiating oligodendroglial progenitor cells isolated from mouse brains in the NG2 positive differentiation state. This is accompanied by higher migration capacity. In line, siRNA-mediated knock-down of glutaredoxin 2 diminished migration capacity. Migration of oligodendroglial progenitor cells into brain lesions is inhibited by repellent signals, e.g. via semaphorin 3a. Interestingly, elevated levels of glutaredoxin 2 show the ability to repeal the inhibitory effect of semaphorin3a. Moreover, elevated concentrations of glutaredoxin 2 protect against oligodendroglial cell death induced by nitric oxide which is increased in lesions. Finally, glutaredoxin 2 inhibits Wnt signaling, one pathway identified for the impaired re-myelination of de-myelinated lesions. Taken together, our findings indicate a beneficial role of glutaredoxin 2 on the re-myelination capacity of oligodendroglial progenitor cells in inflammatory de-myelinating conditions. Consequently, glutaredoxin 2 promotes regeneration in an antibody mediated re-myelination model using cerebellar organotypic slice cultures. *In vivo*, we measured decreased levels of glutaredoxin 2 in the chronic phase of experimental autoimmune encephalomyelitis, the animal model of multiple sclerosis supporting the importance of glutaredoxin 2 for regeneration.

Thus, based on the presented data, we hypothesize an important physiological and feasible therapeutic role of glutaredoxin 2 during progression of diseases associated with neuroinflammation and demyelination such as multiple sclerosis.

T14-19A

The role of NgR and P75NTR on the glia scar formation after traumatic brain injury

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Traumatic brain injury usually results in astrocyte activation, proliferation, formation of reactive astrocyte and the formation of glia scar. The glia scar as a physical and chemical barrier will inhibit axon regeneration and the recovery of neurological function after traumatic brain injury. NgR is identified as serving as a high affinity binding protein for the three classical myelin-associated inhibitors such as Nogo-A, MAG and OMgp, which limit axon regeneration and sprouting in the injured brain. p75NTR forms the receptor complexes with NgR to activate the intracellular signaling pathway, ultimately arresting neurite outgrowth. But what are the role of NgR and P75NTR on the glia scar formation after traumatic brain injury ? It remains poorly understood. In this study, we explored the role of NgR and P75NTR on the glia scar formation after traumatic brain injury in vivo and in vitro. We found NgR and P75NTR could express on the astrocyte. The expression of NgR, P75NTR and CSPG on the astrocytes around injury site were increased obviously until 14 days after stab wound injury. When animals were administered by NEP1-40 in the injury site, astrocyte decreased expression of CSPG or neurocan significantly compared with vehicle group, and the co-expression of CSPG and neurocan with GFAP decreased obviously in p75NTR-/- mice compared with the p75NTR+/+ littermates. We also established the model of glia scar in vitro. The results showed that glia scar

formation was inhibited after cells (co-culture of astrocyte and fibroblast) were treated with NEP1-40 or PI-PLC and the expression of neurocan also decreased significantly. The neurocan expression on the cells from the p75NTR knockout mice obvious decreased compared with cells from wild -type mice. The above results suggest that NgR or P75NTR involved possibly the glia scar formation after traumatic brain injury.

T14-20A

Development of an *in vitro* microfluidic device of spinal cord injury to identify novel compounds for repair

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Spinal cord injury (SCI) has an extremely complex pathology and results in permanent disability. There has been remarkable progress over the past decade in understanding the mechanisms behind lack of central nervous system regeneration after SCI. It is currently thought that combination of pharmacological, rehabilitation and biological approaches are optimal for overcoming SCI. To test this combinational approach however, would utilize large animal cohorts and be time consuming; this has led to the establishment of primary cell co-cultures *that* models aspects of SCI. Recently it has been shown that heparan sulphates (HS) have an important role in activating astrocytosis. Using the *in vitro* model of SCI we have shown that HS can also play a role in repair features of SCI and potentially be a useful therapeutic compound. We investigate if chemical modification of HS with low sulphation levels ($HS\text{-}S^{-}$) may affect SCI repair. $HS\text{-}S^{-}$ influence OPC and astrocytes behavior leading to increased myelination and neurite outgrowth following injury. Our current model is effective but limited as therapeutics and injury cannot be delivered compartmentally. For this reason a novel microfluidic device (MF-SCI) is being developed. MF technology allows precise control over the microenvironment through manipulation of diffusion gradients and compartmentalisation of neuronal cell bodies from neurite projections. We have shown our previous primary cell model of SCI can be incorporated into the MF technology; survive and isolate neuronal cell bodies from neurites/axons projections. The combination of this culture model and the MF technique described presents a novel method for investigation of targeted injury induction to axons and controlled treatment of neuronal populations. Creating a moderate-through put *in vitro* assay which can advance future studies of potential novel compounds in SCI repair.

T14-21A

Differential abilities of acutely and chronically denervated nerve derived and skin derived Schwann cells to support axonal regeneration and remyelination

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Schwann cells (SCs) play a key role in supporting axonal regeneration and remyelination following a peripheral nerve injury. It is well known that outcomes following delayed nerve repair are poorer. Data suggests that in the chronically denervated nerve, SCs progressively lose their capacity to support axonal regeneration and may be less robust for remyelination. We hypothesized that recapitulating the early denervation phenotype of SCs in chronic denervation may restore remyelination and regeneration support capacity. In this study, we compared SCs from adult rodent sciatic nerve with acute and chronic denervation, adult rodent skin derived precursor SCs (SKP-SCs), and nerve derived SCs from E16 embryonic nerve. SCs re-express key pro-myelinating transcription factors (Oct-6 and Krox-20) following acute (day 5) nerve injury, but lose this phenotype with chronic denervation (day 56) both *in vivo* and in cultured nerve SCs *in vitro*. We found that SKP-SCs express Oct-6 and Krox-20*, in vitro*, to similar levels as the ones from acutely denervated nerve and significantly greatly than ones from chronically denervated nerve. We next tested and compared the various SCs for myelination both *in vitro* and *in vivo* and neurite outgrowth assay (DRG-SCs co-culture) *in vitro*. Additionally we compared SKP-SCs and SCs for cellular proliferation, cytokine releasing capacity and immune modulation by macrophage (M2 type) activation. Adult SKP-SCs were comparable to acutely

denervated nerve SCs or embryonic nerve SCs in terms of proliferation, survival in injured nerve, *in vitro* and *in vivo* myelination, *in vitro* neurite outgrowth and immune modulation in injured nerve. Chronically denervated SCs were significantly poorer in all these capabilities. From this study we conclude that: 1) temporal delay following injury results in important phenotypic changes in distal Schwann cells within the nerve and 2) adult SKP-SCs can be used as an alternate therapy to modulate immune response, restore myelination and promote axonal regeneration, in injured peripheral nerve, making these cells a favorable source of autologous cell transplantation. In ongoing work, we are further characterizing the myelinating capacity of SCs from both rodent and human adult skin for myelination and regenerative abilities.

T14-22A

The Merlin tumour suppressor is critical for peripheral nerve regeneration and repair

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The tumour suppressor Merlin has been widely studied for its role in the development of schwannoma tumours in patients with neurofibromatosis type 2, however the putative role of Merlin in the developing nerve, Schwann cell plasticity and peripheral nerve repair has not been investigated.

Schwann cell-specific Merlin loss leads to hypomyelination in the early developing peripheral nerve, but which is corrected by adulthood. Merlin-null nerves exhibit increased Schwann cell proliferation in the developing nerve which results in a decreased internodal length as well as an increased number of Schmidt-Lanterman cytoplasmic channels in the myelinating Schwann cells.

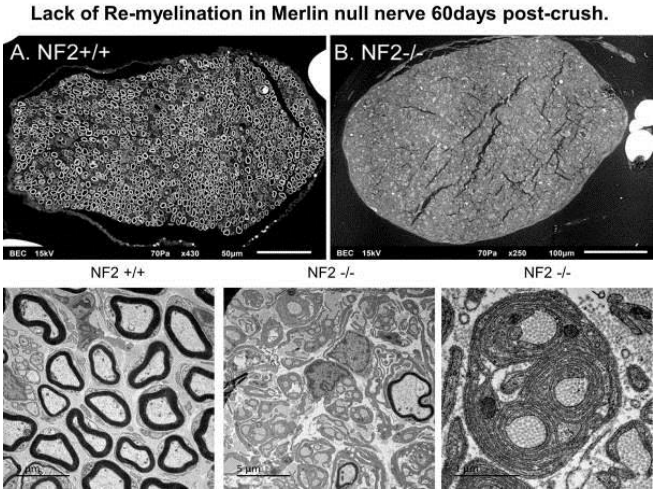
Following peripheral nerve injury, Merlin-null nerves show an increased activation of the ERK1/2 and p38 mitogen activated protein kinases and higher levels of Schwann cell proliferation. Induction of the cJun transcription factor, a known mediator of peripheral nerve repair in Schwann cells, is both delayed and reduced in Merlin-null nerves.

In addition to these findings, we see increased production of the macrophage chemoattractant molecule monocyte chemoattractant protein-1 (CCL2) and increased numbers of macrophages in Merlin-null nerves at all timepoints examined after injury.

We have also compared long-term nerve repair of wild type and Merlin-null adult nerves following peripheral nerve injury and while control nerves repaired normally, Merlin-null nerves repair very poorly. As well as showing ongoing Schwann cell proliferation, Merlin-null nerves show a very limited axonal regeneration, limited remyelination and very impaired functional recovery. We observe continued macrophage presence, increased Schwann cell derived cellularity staining positive for schwannoma markers, nuclear pleiomorphism and collagen type IV deposition. At the cellular level, the Merlin-null Schwann cells extend multiple cytoplasmic processes and exhibit abnormal behaviour by ensheathing bundles of collagen fibres distally to the injury site. Furthermore, we observe an injury-specific activation of components of Hippo/YAP signalling in Merlin-null nerves and are currently investigating the role of this pathway in the phenotype we observe.

These findings demonstrate that Merlin is absolutely required for peripheral nerve repair and that PNS injury may be an initiating event in the generation of Merlin-null schwannoma tumours.

Image



T14-23A

Intraspinal delivery of polyethylene glycol coated gold nanoparticles promotes functional recovery after spinal cord injury

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Failure of the mammalian central nervous system (CNS) to regenerate effectively after injury leads to mostly irreversible functional impairment. Gold nanoparticles (AuNPs) are promising candidates for drug delivery in combination with tissue-compatible reagents, such as polyethylene glycol (PEG). PEG administration in CNS injury models has received interest for potential therapy, but toxicity and low bioavailability prevents clinical application. Here we show that intraspinal delivery of PEG-functionalized 40-nm-AuNPs at early stages after mouse spinal cord injury is beneficial for recovery and superior to free PEG. Positive outcome of hind limb motor function was accompanied by attenuated inflammatory response, enhanced motor neuron survival and increased myelination of spared or regrown/sprouted axons. No adverse effects, such as body weight loss, ill health or increased mortality were observed. We propose that PEG-AuNPs represent a favorable drug-delivery platform with therapeutic potential that could be further enhanced if PEG-AuNPs are used as carriers of regeneration-promoting molecules.

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T14-24A

Control of Schwann cell phenotype after nerve injury

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Myelinating Schwann cells trans-differentiate into a specialized repair-promoting phenotype (the Bungner cell) after nerve injury. This process is mediated by the AP-1 transcription factor c-Jun. Here we explore how c-Jun levels are regulated using both sciatic nerve-explants and Schwann cells in culture. Our results suggest that a drop in the cAMP levels in the injured nerve activates the expression of this transcription factor and the trans-differentiation program. Loss of function experiments shows that cAMP effects are mediated by the translocation of histone deacetylase 4 (HDAC4) into the nucleus of Schwann cells. On the other hand, gain of function experiments (using the mutant HDAC4 3SA) demonstrate that this translocation is enough to block c-Jun expression and to switch-on the differentiation program in Schwann cells. We also show that the effect of HDAC4 on c-Jun expression is mediated by its capacity to recruit the deacetylase activity of HDAC3 through the NcoR1-SMRT family of proteins. We propose a model in which HDAC4 senses intracellular cAMP levels and translocate into the nucleus acting as a brake to repress the expression c-Jun and prevent the trans-differentiation of the myelinating Schwann cell into a Bungner repair cell.

T14-25A

De novo expression of parvalbumin in ependymal cells in response to brain injury promotes ependymal remodeling and wound repair

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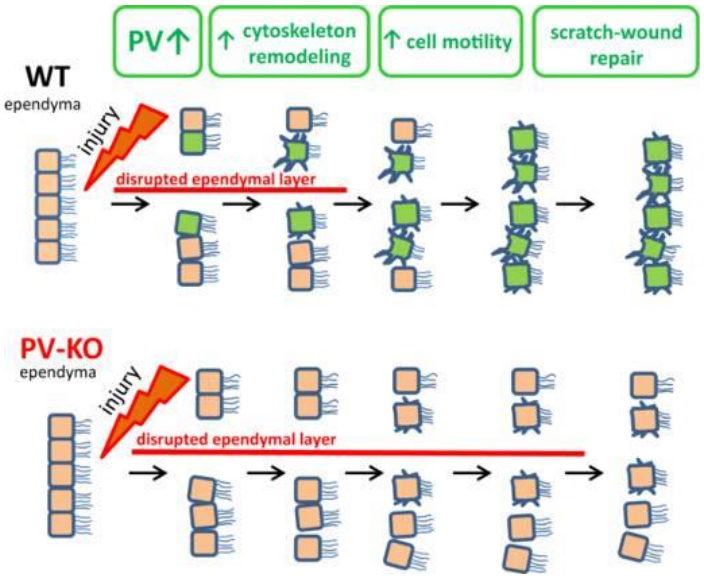
The calcium-binding protein parvalbumin (PV) hallmarks subpopulations of interneurons in the murine brain. We serendipitously observed the *de-novo* expression of PV in ependymal cells of the lateral ventricle wall following *in-vivo* lesioning and brain slicing for the preparation of organotypic hippocampal slice cultures (OHSCs). In OHSCs, *de-novo* PV-expression begins shortly after the onset of culturing, and the number of ependymal cells implicated in this process increases with time. PV-immunopositive ependymal cells aggregate and form compact cell clusters, which are characterized by lumen-formation and beating cilia. Scratches inflicted on such clusters with a sharp knife are rapidly closed. Exposure of OHSCs to NF-KB-inhibitors and to antioxidants reduces PV-expression in ependymal cells, thereby implicating injury-induced inflammation in this process. Indeed, *in-vivo* stab injury enhances PV-expression in ependymal cells adjacent to the lesion, whereas neuraminidase denudation is without effect. PV-knock-out mice manifest an impaired wound-healing response to *in-vivo* injury, and a reduced scratch-wound reparation capacity in OHSCs. Whole-transcriptome analysis of ependymal-cell clusters in OHSCs revealed down-regulation of genes involved in cytoskeletal rearrangement, cell motility and cell adhesion in PV-knock out mice as compared to wild-type mice. Our data indicate that the injury-triggered up-regulation of PV-expression is mediated by inflammatory cytokines, and promotes the motility and adhesion of ependymal cells, thereby contributing to leakage closure by the re-establishment of a continuous ependymal layer.

Image



T14-26A

Light activation of astrocytes promotes neuronal differentiation of stem cells and improves neurological deficit in stroke rats

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Question: Astrocytes have been identified as key components of the stem cell niche. However, it is not clear whether astrocyte-derived ATP plays a vital role in modulating the function of mesenchymal stem cells (MSCs).

Methods and Results: we co-cultured MSCs with light-stimulated-channelrhodopisn-2 (ChR2)-astrocytes, and observed these MSCs expressed more neuronal markers, Tuj1 and NeuN. Furthermore, the ChR2-astrocyte-conditioned medium markedly up-regulated mRNA expression of Tuj1 and Pax6, and promoted the DNA synthesis of MSCs, indicating some component(s) from the photostimulated ChR2-astrocytes contributed to the differentiation- and proliferation-enhancing effects. Optical stimulation of ChR2-astrocytes significantly increased ATP accumulation in their bathing medium without impairing the cell membrane. We further demonstrated the enhancing effects of ATP on the MSCs through the wnt/β-catenin signalling in a dose-dependent manner. Furthermore, either FZD8 or β-catenin mRNA level was significantly increased by ATP, and this effect could be reversed by application of the selective P2X receptor antagonist, TNP-ATP. Finally but importantly, our study also demonstrated that light-controlled astrocytes stimulated endogenous ATP release into the ischemic area to influence the transplanted MSC-niche, resulting in steering the MSCs towards neuronal differentiation and improvements of neurological deficit in the stroke rats.

Conclusions: Together these data provide convergent evidence that ATP from photostimulated-astrocytes, through binding to the P2X receptors expressed by MSCs, activates the wnt/β-catenin signalling, and as a consequence, upregulates neuronal differentiation of MSC both in vitro and in vivo, thereb providing a molecular mechanism for modulation of stem cell function by activated-astrocytes within a special niche.

T14-27A

Comparison of HDAC functions in oligodendrocyte and Schwann cell plasticity after axon injury

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The peripheral nervous system (PNS) is able to regenerate after a lesion, whereas regeneration of the central nervous system (CNS) is mostly inefficient. Efficiency of regeneration in the PNS after lesion is mainly due to the remarkable plasticity of Schwann cells, the myelinating glia of the PNS. After a nerve lesion, Schwann cells dedifferentiate, proliferate and migrate along damaged axons to foster their regrowth. Dedifferentiated Schwann cells can then redifferentiate and remyelinate regenerated axons. In contrast, oligodendrocytes, the myelinating glia of the CNS, do not have this ability. Because Schwann cell plasticity involves rapid and dynamic regulation of many genes, our research is focused on histone deacetylases (HDACs), which can potentially translate an external stimulus such as a lesion into chromatin remodeling and thereby regulate gene expression. In addition, our group has found that several members of the HDAC family are strongly regulated in Schwann cells after lesion during regeneration, suggesting important functions in this process.

The aim of my project is to identify differences of HDAC regulations and functions in Schwann cells and oligodendrocytes during regeneration after a lesion and determine how these differences affect their plasticity after a lesion.

T14-01B

PDGFRα-positive progenitor cells form myelinationg oligodendrocytes and Schwann cells following contusion spinal cord injury

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Question: Contusive spinal cord injury (SCI) results in considerable demyelination of spared axons, which impairs signal transduction and may leave axons vulnerable to degeneration. Both oligodendrocytes (OL)s and Schwann cells remyelinate denuded axons in the subsequent weeks and months following SCI. NG2 cells, characterized by the near ubiquitous co-expression of platelet derived growth factor receptor α (PDGFRα) in the uninjured central nervous system (CNS), are oligodendrocyte progenitors (OP)s which may serve as a source of new OLs following SCI. Previously, Zawadzka et al. (2010) demonstrated that the Schwann cells within chemical demyelination lesions are derived from OPs. Importantly, understanding whether the Schwann cells are migrating in from the periphery or whether they are derived from OPs in the injured spinal cord is an important question considering that these cells contribute to repair after injury.

Methods: PDGFRα-CreERT mice were crossed with Rosa26-YFP mice and administered tamoxifen to label OPs two weeks prior to contusive thoracic spinal cord injury.

Results: In the uninjured spinal cord we found that YFP was expressed in NG2+ OPs at very high efficiency, as well as αSMA+ pericytes and fibronectin+ fibrocytic cells in the spinal roots. Following injury, many recombined cells continue to express the PDGFRα+, Olig2 and NG2, indicative they have remained as OPs, but substantial differentiation into new mature oligodendrocytes (CC1+) was observed, responsible for de novo ensheathment of >30% of the myelinated axons by three months. Strikingly, the majority of P0+ Schwann cells in the spinal cord expressed YFP, suggesting they originated from central nervous system PDGFRα+ OPs. Further work is currently underway to corroborate these fate mapping data with an olig2-CreERT:Rosa26-YFP mouse line and to assess whether other cells contribute to the Schwann cell population within the injured cord. In addition, P0-

positive myelinating Schwann cells are being fate mapped to investigate the relative contribution of migrating Schwann cells originating in the periphery.

Conclusions: Overall, this work reveals enormous phenotypic plasticity of PDGFRα precursors following spinal cord injury as a source of the new remyelinating Schwann cells and oligodendrocytes in the injured spinal cord.

This work is supported by the Canadian Institute of Health Research, and the Multiple Sclerosis Society of Canada.

T14-02B

Human olfactory derived mesenchymal stem cell transplantation as a candidate for CNS repair

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Previously we have identified and characterised a source of adult MSCs from the uniquely regenerative human olfactory mucosa (OM-MSCs) and shown that they have enhanced clonogenicity, proliferation rate and *in vitro* CNS myelination compared to bone marrow derived MSCs (BM-MSCs). For comparison of the two MSC types, microRNA-based (miRNA) fingerprinting was carried out, which demonstrated 64% homology between the two MSC types. Interestingly, 26 miRNAs were differentially expressed, and of these, we focussed on miR-146a-5p due to its reported role in the regulation of chemokine production. We found that CXCL12, a chemokine regulated by this microRNA was differentially secreted by OM-MSCs. Addition of CXCL12 to myelinating cultures promoted myelination and a selective CXCL12 receptor blocker and anti-CXCL12 prevented the promyelinating effect. Transduction with the miR-140-5p antagomir and mimic produced inverse changes in CXCL12 RNA, confirming the regulatory role of miR-140-5p for CXCL12. To assess the repair potential of human OM-MSCs GFP tagged-cells were transplanted 3 weeks post-injury into the spinal cord of rats with a thoracic level 9 injury. Cells filled the lesion surviving until at least 4 weeks post-transplant and resulted in reduced levels of cavitation, and a greater amount of neurofillament positive fibres within the lesion site. Data obtained using a treadmill based gait analysis system suggested that transplanted animals recovered co-ordinated stepping earlier than control animals with immunohistochemical assessment revealing enhanced peripheral myelination within the cavity and within ventral and lateral areas of the cord by invading Schwann cells. These data suggest that OM-MSCs promote both de novo myelination and re-myelination of injured/spared axons which could account for the quicker restoration of co-ordinated stepping that was found within transplanted animals. Thus tissue niche may play an important role in determining how beneficial a particular MSC type might be in terms of SCI repair and that OM-MSCs which promote myelination could be a better MSC choice for use in the clinic.

T14-03B

Dual effect of salubrinal after a cortical stab wound injury in mice

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After CNS injury, astrocytes and mesenchymal cells secrete proteoglycans and extracellular matrix proteins (ECMs), forming the so-called glial scar, a simulation of the disrupted *glia limitans*. Although the glial scar is important in sealing the lesion, it is also a physical and functional barrier that prevents axonal regeneration.

The synthesis of secretory proteins in the RER is under the control of the initiation factor of translation eIF2α. Inhibiting the synthesis of secretory proteins by increasing the phosphorylation of eIF2α, might be a pharmacologically efficient way of reducing proteoglycans and other profibrotic proteins present in the glial scar, and modulating its formation. Salubrinal, a neuroprotective drug, decreases RER

protein translation by maintaining eIF2alpha phosphorylated. In previous studies, we demonstrated that Salubrinal promotes the neurite outgrowth in an *in vitro* model of glial scar.

Acute treatment with Salubrinal after a stab injury in the cerebral cortex reduced glial scar markers, but increased fibronectin expression around the injury site. However, chronic Salubrinal treatment increased astroglial reactivity. Therefore, the effect of Salubrinal on glial reactivity and fibrosis depended on the postlesion time at which the animals were treated. Acute treatment with Salubrinal also reduced blood leakage to the CNS parenchyma, increasing neuronal survival in the lesion area.

These contradictory results indicate that the state of eIF2a phosphorylation is a key factor regulating inflammation and fibrosis after CNS injury.

T14-04B

STAT3 is required for the long-term maintenance of the repair Schwann cell phenotype in injured nerves

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The transcription factor STAT3 is implicated in a wide variety of biological processes including proliferation, survival and differentiation. After nerve injury STAT3 is activated in the axons of PNS neurons in response to cytokines, growth factors and hormones at the injury site and retrogradely transported to the cell body where it activates genes involved in axon regeneration and neuronal survival. So far, the role of this factor in Schwann cells of injured nerves has not been extensively investigated.

In the distal stump of injured nerves, the loss of axonal contact triggers the reprogramming of myelin and non-myelin (Remak) Schwann cells to form repair (Bungner) Schwann cells. These cells break down myelin by activation of autophagy and macrophage recruitment, express high levels of factors that promote neuronal survival and regeneration and form regeneration tracks (Bands of Bungner) that direct axons back to their targets. In larger animals including man, the Bungner cells in more distal parts of regenerating nerves are deprived of axonal contact for a long time as axonal regeneration progresses more proximally. These long-term denervated Schwann cells gradually lose their ability to support axon growth and eventually die. This is a major reason for the poor recovery after nerve injury in humans. Thus, mechanisms that ensure long-term Schwann cell survival and maintenance of the repair phenotype in injured nerves are critical for nerve repair.

Our findings reveal that nerve injury results in STAT3 phosphorylation on Tyrosine705 and Serine727 residues in the Schwann cells in the distal nerve stump, and that the survival of Schwann cells in long-term denervated distal stumps is markedly reduced in mice with conditional deletion of STAT3 in Schwann cells. The role of STAT3 in Schwann cell survival was confirmed and analysed in vitro. STAT3 is also required for the long-term maintenance of structurally normal regeneration tracks (Bands of Bungner), while regeneration in short-term experiments is normal in mice without Schwann cell STAT3.

We suggest that while c-Jun is required for the initial generation of repair Schwann cells (Arthur-Farraj et al., 2012), STAT3 is required for the prolonged maintenance of the repair phenotype and for the long-term survival of the Bungner repair cells. STAT3 signalling is therefore a potential target for improving nerve regeneration.

Reference:

Arthur-Farraj *et al*., (2012); c-Jun reprograms Schwann cells of injured nerves to generate a repair cell essential for regeneration, Neuron 75:633-647.

T14-05B

Control of Oligodendrocyte Precursor Cell function by their microenvironment

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Demyelination of neuronal axons leading to neurodegeneration causes permanent disability in Multiple Sclerosis (MS). A major goal of regenerative therapy is therefore to promote repair of the degraded myelin sheath so protecting axons. Oligodendrocyte precursor cells (OPCs) carry out myelin repair and their proliferation, migration and differentiation are crucial steps in remyelination. We hypothesise that the OPC microenvironment is primarily responsible for regulating OPC function and is thus a target for future therapeutic approaches in MS.
We employed an in vitro approach to investigate the role of known extracellular matrix (ECM) molecules in regulating OPC function and identified effects of laminin, decorin and fibronectin on OPC viability, proliferation and differentiation using manual cell counting. In order to study the effects of different doses and combinations of ECM molecules typically found in the brain, we have for the first time developed a high-throughput method for imaging and analysis of OPCs using the Operetta automated imaging system with Columbus software.
We also employed an in vivo mouse model for focal demyelination to identify components of the cellular and extracellular OPC microenvironment during de- and remyelination by high resolution imaging. We found that OPCs directly interact with laminin containing blood vessels in the vicinity of demyelinated lesions and that the ECM molecule decorin is highly expressed in cells in and around demyelinated lesion sites.
The results of our in vitro and in vivo studies point at profound effects of the ECM on OPC functions necessary for myelin repair. We have identified changes in the OPC microenvironment in response to demyelination in vivo which also affect OPC function in vitro. Future studies will expand on this knowledge to reveal the detailed molecular changes that OPCs are exposed to during demyelination and repair and therefore identify new targets for promoting myelin regeneration in MS.

T14-06B

Essential role of endogenous fatty acid synthesis in CNS myelin regeneration

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Remyelination requires adult oligodendrocyte progenitor cells (OPCs) to proliferate and subsequently differentiate into myelinating cells, hence calling for a tremendous surge in lipid availability. Fatty acids are the primary constituents of cellular membrane lipids, and thus myelin itself. Furthermore, fatty acids are critical towards a variety of fundamental cellular functions, including membrane targeting of proteins, energy storage, cell signalling and transcriptional regulation. While most cells are thought to mainly rely upon uptake to maintain their fatty acids pool, highly metabolically active and proliferative, i.e. cancer and stem cells are strongly functionally dependent upon *de novo* synthesis, mediated by fatty acid synthase (Fasn). Fasn primarily catalyses the synthesis of the saturated fatty acid palmitate, from acetyl-CoA, malonyl-CoA and NADPH. The importance of this fundamental metabolic pathway during CNS remyelination has so far not been fully clarified. Thus, we addressed the functional role of de novo fatty acid synthesis in adult OPC-mediated remyelination. Using inducible Cre/lox system, we examined the effect of conditional depletion of Fasn in adult OPCs on remyelination, following lysolecithin-induced demyelination. We show that Fasn-mediated *de novo* fatty acid synthesis is critical to achieve timely and efficient CNS remyelination.

T14-07B

A co-culture system to study interactions between sympathetic neurons and glial progenitors

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The nervous system, despite its important interactions with virtually every organ system of the body, possesses little or no capacity to repair itself and recover from damage. This compromised regenerative ability leads to diminished mobility and often complete loss of neuromuscular function, motor control, and conducting activity (Collinger et. al., 2013). We have begun to examine how neurons and glia influence the integrity and state of differentiation of one another when co-cultured in proximity. Our system involves sympathetic adrenal medullary (PC12) cells and immortalized neural crest stem cells (NCSCs) that resemble immature peripheral glia. Analyses of cultures using immunostaining, morphometry and comparisons of gene expression have provided insight into factors that may influence the capacity for regeneration following nerve trauma. Our glial progenitors express GFAP, the early transcription factor Krox20 and exhibit an elongated morphology following induced differentiation that are $GFAP^-$ and MBP^+. The PC12 cells adopt a more stellate, branched appearance with robust NF-M, and detectable transcripts for Sox11, NURR and NRTK1. Using spatial and temporal patterns of expression of these and other lineage markers, we can analyze our complex culture systems after varying the state of differentiation, changing the timing at which the co-cultures are formed, as well as isolating single cell populations post co-culture. It is our hope that these experimental designs might recapitulate in part, the types of contacts and interactions present within an injury environment. With this relevant developmental model we will mimic and further characterize the nervous system response to injury and identify if perhaps there is an optimal combination or ratio of immature or mature glia to neurons that is required to promote or limit healing of a nerve.

T14-08B

Schwann cell dynamics and function during peripheral nerve regeneration

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In the peripheral nervous system, axons damaged by injury have the ability to regrow and reconnect to their original targets. Schwann cells are critical for axonal maintenance and regeneration and following injury, Schwann cells dedifferentiate to an immature state that promotes axonal regrowth and nerve repair. The process of Schwann cell dedifferentiation is accompanied by drastic morphological changes as well as cell migration. However, exactly how these morphological changes are driven by intracellular events, such as cytoskeletal rearrangements and membrane remodeling via intracellular transport, remains unknown.

We have recently established an *in vivo* assay to laser-transect GFP-expressing motor nerves in live intact zebrafish larvae, and to subsequently observe at a minute-by-minute resolution the processes of axonal de- and regeneration (Rosenberg et al 2012). Using this assay, I am determining how cytoskeletal and intracellular transport dynamics in Schwann cells change in response to nerve injury. By expressing various fluorescently tagged cytoskeletal and intracellular transport proteins in Schwann cells, I have been able to visualize and quantify Schwann cell cytoskeletal and intracellular transport dynamics in vivo. Ongoing experiments continue to characterize how the cytoskeleton and intracellular transport dynamics in Schwann cells respond to nerve injury. In order to probe the requirement for intracellular transport in axonal regeneration, I assayed regeneration in mutants for the motor protein dynein. I found that both homozygous but also heterozygous dynein mutants exhibit impaired axonal regrowth, indicating a requirement for dynein during axonal regrowth *in vivo*. Recent studies have shown that dynein is required in Schwann cells for proper differentiation and myelination during development. This suggests that besides its well-established role in neurons, dynein may also play an important role in Schwann cells after injury and during axonal regeneration. I am using complementary cell-type specific knockdown and rescue approaches to test whether dynein is required in Schwann cells during axonal regeneration. I will present results of these ongoing experiments to determine the role of cytoskeletal rearrangements and intracellular transport in Schwann cells after nerve injury.

T14-09B

Human Schwann-like adipose-derived stem cells combined with synthetic biodegradable polymer scaffolds for nerve regeneration

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Introduction: Peripheral nerves have capacity to regenerate after injury due to the Schwann cells (SC) response; however, functional outcomes remain unsatisfactory in particular in the presence of a nerve gap, and regenerative medicine approaches are sought to improve the neural response. Our strategy aims to combine the use of regenerative cells and synthetic biodegradable scaffolds for the creation of bio-artificial conduits able to form a favorable environment for nerve re-generation. One of the challenges of this approach is to find a suitable source of easily accessible, rapidly expandable and highly regenerative, transplantable cells to mimic SC action.

Methods: We have trans-differentiated human adipose-derived stem cells (ASC) towards SC-like ASC and measured morphology and gene expression (qRT-PCR) of key growth factors at 0/7/14 days of the treatment protocol. Finally we utilized a co-culture model of rat dorsal root ganglia neurons and SC-like ASC on poly e-caprolactone and polylactic acid blended films, with engineered surface topography, to examine the patterns of neurite regeneration.

Results: SC-like ASC demonstrated elongated morphology compared to native SC. We found several neuroglia associated genes to be strongly upregulated following glial differentiation: Nestin, 48±16 fold increase (n=4, *P<0.05); Brain derived neurotrophic factor, BDNF, 2.03±0.32 fold increase (n=4, *P<0.05); Glial derived neurotrophic factor, GDNF, 4.93±1.41 fold increase (n=3, *P<0.05). Conversely, other genes that are related to neuron-glia interactions were strongly down-regulated: Nerve growth factor, NGF expression was reduced by 60% (n=4, ***P<0.001); Neuregulin 1, NRG-1 expression was reduced by 70% (n=3, ***P<0.001); Epidermal growth factor receptor 3 expression, ErB-B3, a receptor for NRG-1, was reduced 60% (n=3, ** P<0.01); Neurotrophin 3, NT-3 expression was almost completely abolished >90% (n=3, ****P<0.0001).

SC-like ASC were successfully grown on the polymers and the topographical cues were found to improve cell morphology and growth rate. Dissociated dorsal root ganglia were added to polymer films pre-seeded with SC-like ASC and the patterned films were found to positively/preferentially direct axonal growth.

Conclusion: this study provides in vitro evidence that human SC-like ASC can be combined with engineered biodegradable polymer scaffolds towards improved nerve regeneration.

T14-10B

Schwann cells in the proximal stump of injured nerves activate c-Jun to control the intrinsic growth state and regeneration potential of DRG sensory neurons

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Following peripheral nerve damage, up-regulation of Schwann cell c-Jun in the nerve stump distal to the injury is crucial for the conversion of myelin- and non-myelin (Remak) cells to functionally effective repair (Bungner) Schwann cells (Arthur-Farraj et al., 2012).

Here we show that c-Jun is also activated in Schwann cell nuclei proximal to the injury site, where ~70% of nuclei are c-Jun positive as early as 1 hour after nerve transection. Expression is highest within the first 2mm nearest to the injury, and does not extend beyond 7mm. Elevated c-Jun expression lasts for at least 48 hours following nerve transection.

After nerve injury, dorsal root ganglia (DRG) sensory neurons activate a plethora of regeneration-associated genes (RAGs), switch from a signaling mode to growth mode and regenerate their axons. Using mice with conditional inactivation of c-Jun in Schwann cells only (Arthur-Farraj et al., 2012), we

found that in the absence of c-Jun in the proximal stump Schwann cells, injury-induced expression of key RAGs in DRG cell bodies was reduced. This included c-Jun, GAP43, p-STAT3 and ATF3. This was apparent as early as 6 hours after nerve injury, and more pronounced by 48 hours.

To test the functional relevance of this finding, we examined the ability of DRG neurons to grow within the injured CNS. In this paradigm, conditioning lesion of the sciatic nerve of wild type mice activates the growth mode of DRG neurons and allows their central branch to sprout into spinal cord lesions (Neumann and Woolf, 1999). Strikingly, we found that this regenerative response was absent in mice without c-Jun in proximal stump Schwann cells.

Taken together, these observations indicate that c-Jun dependent signaling between the Schwann cells of the proximal stump and injured axons takes part in controlling the growth state of DRG neurons and determines the ability of these cells to regenerate after injury.

T14-11B

Bridging the gap in spinal cord injury using novel super-macroporous polymer scaffolds

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Spinal cord injury (SCI) is a devastating cause of persistent and often permanent disability. Cell transplantation into the injury site is a promising therapeutic avenue to fill the gap and promote regeneration. Evidence has shown that a range of cell types can be used for transplantation, including glial, neural and genetically engineered cells. One glial cell type, the olfactory ensheathing cell (OEC) is a particularly fitting candidate for transplant-mediated repair due to its inherent ability to support regeneration in the olfactory system throughout life. While transplantation of OECs has been demonstrated as a feasible and safe approach in both humans and animal models, axonal growth is often disorganised, rarely exiting the graft. Moreover, this approach is unfeasible in large human spine lesion sites, where injury is extensive and cells are unable to form a robust, supportive environment to promote organised regeneration. Transplantation of supportive scaffolds alongside cells can bridge the gap commonly formed following SCI and alleviate the chaotic nature of this environment, guiding organised growth. Our lab has previously investigated ε-polycaprolactone (PCL) scaffolds, for this purpose, however they proved too rigid and inflexible for spinal transplantation. Here we introduce an artificial macroporous scaffold, Proliferate®, invented by SpheriTech Ltd, with potential applications in SCI and general CNS repair. Using primary cultures which mimic the intact CNS, we have shown the novel super-macroporous polymer scaffolds, designed to promote well-organised and appropriately aligned axonal growth, are capable of supporting OECs and other glial cell types *in vitro*. These scaffolds are biocompatible, and are currently under development for regenerative medicine including wound healing, retinal and corneal repair, bone and cartilage repair, and peripheral nerve regeneration. Data will be presented on optimisation of these materials to support growth, proliferation and differentiation of glia and neurons *in vitro* as a prerequisite for transplantation *in vivo*. By optimising this scaffold for spinal repair we hope to highlight its broader potential applications in pathological and functional recovery of the damaged CNS.

T14-12B

The role of GSK3β in regulating astrogliosis

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CNS injury results in a characteristic reactive gliosis, whereby astrocytes and NG2-glia (oligodendrocyte precursor cells, OPC) undergo cellular hypertrophy and proliferation. The resultant glial scar is both a chemical and physical barrier to axon growth and is one of the primary reasons why

axons in the adult CNS do not regenerate following injury. The mechanisms regulating reactive gliosis and glial scar formation are not fully elucidated, but numerous extracellular trophic signals are implicated. The enzyme glycogen synthase kinase beta (GSK3β) is a key regulator of multiple cellular pathways, including cellular proliferation and migration, and promotes neuronal survival and neurite outgrowth *in vitro* and axonal sprouting following spinal cord injury (SCI). Here, we examine the role of GSK3β in astrogliosis and scar formation using the GSK3β inhibitor AR-A014418 *in vitro* in astrocyte cell cultures and *ex vivo* in organotypic spinal cord slice cultures. In an *in vitro* 'scratch assay' of the astrocyte response to injury, there was complete closure of the 'wound' after 50h in control medium and this was almost blocked by treatment with AR-A014418. Western blot analysis also indicated AR-A014418 altered expression lysyl oxidase (Lox), consistent with Lox being a key regulator of astrocyte morphology and a target of GSK3β signalling. The morphogenic effects of AR-A014418 were confirmed *ex vivo* in spinal cord slice cultures from GFAP-EGFP mice. Notably, preliminary data indicate that AR-A0114418 promote the generation of cells that expressed the GFAP-EGFP reporter and were immunopositive for NG2. Our data support a central role for GSK3β and Lox in regulating astrogliosis.

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T14-13B

Daam2-PIP5K is a novel regulatory pathway for Wnt signaling and therapeutic target for remyelination in the CNS

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Wnt signaling plays an essential role in developmental and regenerative myelination of the CNS, however contributions of proximal regulators of the Wnt receptor complex to these processes remain undefined. To identify components of the Wnt pathway that regulate these processes, we applied a multifaceted discovery platform and found that Daam2-PIP5K comprise a novel pathway regulating Wnt signaling and myelination. Using dorsal patterning of the chick spinal cord we found that Daam2 promotes Wnt signaling and receptor complex formation through PIP5K-PIP_2. Analysis of Daam2 function in oligodendrocytes (OLs) revealed that it suppresses OL differentiation during development, after white matter injury (WMI), and is expressed in human white matter lesions. These findings suggest a pharmacological strategy to inhibit Daam2-PIP5K function, application of which stimulates remyelination after WMI. Put together, our studies integrate information from multiple systems to identify a novel regulatory pathway for Wnt signaling and potential therapeutic target for WMI.

T14-15B

Deciphering mechanisms by which olesoxime promotes oligodendrocyte maturation and remyelination

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Multiple sclerosis (MS) is a neurodegenerative disease characterized by episodes of immune attack and oligodendrocytes death leading to demyelination and progressive functional deficit. One therapeutic strategy could consist in stimulating the spontaneous regenerative process observed in some patients and to favor oligodendrocyte maturation at the lesion site. We have explored the potential effect of olesoxime, a cholesterol like compound (Bordet et al. J Pharmacol Exp Ther 2007), on neuroprotection and remyelination. We have demonstrated that the compound directly promotes OPC maturation and myelination in a variety of in vitro assays while it has no effect on OPC survival or proliferation. We also showed that olesoxime can promote remyelination and favour functional recovery in vivo, using two distinct demyelination models. Altogether we have provided the final proof

of concept that this compound promotes remyelination and favors neuroprotection in animal models of MS (Magalon et al. Ann Neurol. 2012). Olesoxime is currently under Phase Ib/IIa clinical study for the treatment of MS. However, the precise mode of action of olesoxime on oligodendrocyte is not clear a shortcoming that could be detrimental for further clinical use.

Using purified primary OPCs we first showed that olesoxime binds specifically to mitochondria during processes extension. Knowing that mitochondrial function is a key regulator of oligodendrocyte differentiation in vitro, we explored mitochondrial distribution and metabolism in OPCs after olesoxime treatment. We observed an increase of the length of mitochondria in OPCs without any change in mitochondrial biogenesis. This could suggest an effect on the regulation of fission/fusion mechanisms that are tightly lying to mitochondria activity. Our preliminary data could suggest that olesoxime could modulate ROS production acting as an antioxidant. Then, we demonstrated that olesoxime favors process branching by analyzing shape of differentiating oligodendrocytes in culture. We were able to show that olesoxime acts on microtubule dynamics by stimulating EB1 accumulation at growing ends of microtubules. Altogether our results suggest that olesoxime could favor oligodendrocyte maturation by acting on both mitochondria activity and/or cytoskeleton reorganization. The possible link between ROS production and microtubules reorganization is currently under investigation.

T14-16B

Clonal oligodendrocyte progenitor cell dynamics in spinal cord remyelination

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Remyelination, a spontaneous regenerative process, arises from oligodendrocytes newly generated from oligodendrocyte progenitor cells (OPCs) that proliferate and migrate towards and within the site of injury. In this study, OPC clonal dynamics were tracked using the Confetti reporter construct following induction of Cre recombinase under Sox10 and platelet-derived growth factor receptor alpha (PDGFRA) promoters. Consistent with previous studies, we found infrequent turnover of OPC with normal adult white matter. However, following chemically-induced focal demyelination, our data supports a model in which there is proliferation of OPCs in the vicinity of the lesion forming radial clonal clusters near the lesion edge. OPCs then enter the lesion core by transverse migration. By mapping the clonal distribution, we will be able to construct a precise model of how OPCs respond to demyelination and generate replacement oligodendrocytes, and thereby better understand OPC dynamics at a population and single cell level during remyelination.

T14-17B

Microglial changes at the base of a diminished regenerative potential in the aged zebrafish retina

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As adult mammals lack the capacity to regenerate damaged neurons, dysfunction of the central nervous system (CNS) after brain injury or in neurodegenerative diseases increasingly impairs life quality in our aging society. Despite intensive research, induction of axonal regeneration and subsequent functional recovery of the diseased mammalian CNS remains a challenge, especially in an senescent environment. In contrast to mammals, adult zebrafish have a high regenerative capacity. As they were recently identified to age gradually, they form an ideal model to study the effects of aging on regeneration potential and to identify underlying mechanisms. We focus on the zebrafish retinotectal system, a powerful system to study neuronal survival and axonal regrowth after damage.

In a first approach we wished to unravel the contribution of acute inflammation to successful axonal regeneration in the injured retinotectal system of adult zebrafish. Thereto, fish were subjected to optic nerve crush (ONC) and axon outgrowth was followed using biocytin tracing. After ONC, a timed induction and resolution of activated microglia/macrophages, visualized using appropriate immunostainings or the transgenic fish line *Tg(coro1a:eGFP; lyz:dsRed)*, was observed in the retina, optic nerve and tectum. Surprisingly, intravitreal injection of the inflammatory compound zymosan, resulted in an accelerated tectal reinnervation after ONC, indicating that, comparable to mammals, axon regeneration can be stimulated by an induced inflammatory response.

Next, we investigated whether a senescent cellular environment affects neuronal survival and axonal regeneration in aged zebrafish. Detailed immunohistochemical analyses of old zebrafish eyes confirmed an age-related retinal atrophy and revealed an increased number and altered distribution of microglia/macrophages, indicative for a clear manifestation of chronic inflammation or 'i*nflammaging*' in the aged fish retina. Despite this augmented inflammatory status in the old retina, we observed a significant delay in axonal regeneration after ONC, resulting in a reduced reinnervation of the aged tectum. Preliminary data suggest that altered morphological and functional changes of senescent microglia underlie a diminished regeneration capacity in the aged zebrafish CNS.

Further characterization of the acute and chronic inflammatory responses and identification of underlying molecules might unveil new targets for the development of novel regenerative strategies in the senescent mammalian CNS.

T14-18B

Transduction of an immortalized olfactory ensheathing glia line with the green fluorescent protein (GFP) gene: evaluation of its neuroregenerative capacity

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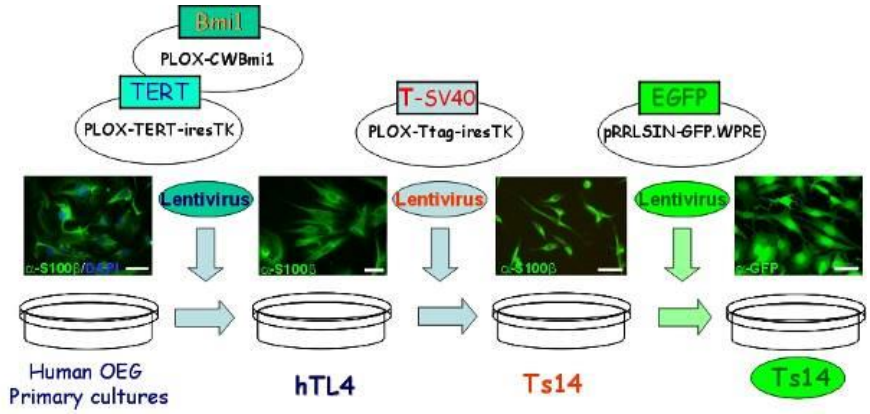
One of the classic problems of the molecular and cellular neurobiology, known from Cajal's studies and yet not solved, is how to modify the poor regeneration of central nervous system (CNS) neurons. During the last few years several studies, both in culture and *in vivo,* have determined that olfactory ensheathing glia (OEG) has properties as potent mediator of axonal regeneration in the CNS. Moreno-Flores et al. established and characterized several clonal lines of reversibly immortalized human OEG (ihOEG) that enabled to develop models for their validation, *in vitro* and *in vivo*.

In this work our aim was to assess the neuroregenerative ability of immortalized olfactory ensheathing glia after viral transduction of GFP. For this, we have used a lentiviral vector with the GFP gene sequence that was packaged and used to infect a previously described human immortalized olfactory ensheathing glia cell line (hiOEG-Ts14). GFP positive cells were selected using fluorescence-activated cell sorting (FACS) and used to perform a coculture assay with adult rat retinal ganglion neurons (RGN). RGN axonal regeneration was analyzed using immunofluorescence, confocal microscopy and computerized image analysis. The percentage of RGN with an axon and the mean axonal length were quantified by using ImageJ software (NeuronN plugin).

A constitutively GFP expressing hiOEG cell line was obtained. The neuroregenerative properties of this cell line were similar to those found for the parental line Ts14 and for other hiOEG previously tested. Therefore, Ts14-GFP cell line expresses GFP constitutively, induces neuroregeneration and can be assayed in *in vivo* transplant experimental models, afterCNS damage (i.e. spinal cord and optic nerve injuries).

Image



T14-19B

The role of Hippo/YAP signalling in Schwann cell development and peripheral nerve repair

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Schwann cells are the main glial cells of the peripheral nervous system (PNS) and myelinate neurons in a one-to-one relationship. The signals that control Schwann cell proliferation during both development and after injury are not yet fully understood, which led us to investigate the role of the Hippo signalling pathway. The conserved Hippo signalling pathway has been shown to regulate organ size in both *Drosophila* and mammals. In mammalian cells, YAP and its transcriptional co-activator TAZ are found downstream of the Hippo pathway kinase cascade, activation of which causes their inhibition via phosphorylation and cytoplasmic retention. Using a Schwann cell-specific knockout of the YAP effector of the Hippo pathway we have studied the role of this protein in Schwann cell development and myelination in vivo. Furthermore, we are studying the role of the YAP protein in the processes of peripheral nerve repair following injury. Loss of the tumour suppressor Merlin causes, in many cell types, activation of the Hippo signalling pathway and nuclear localisation of the YAP protein. Loss of Merlin in Schwann cells causes a large increase in Schwann cell proliferation and defects in axonal regeneration after injury. We are currently investigating the role of Hippo signalling and the YAP protein in these effects.

T14-20B

Intralesional transplantation of mesenchymal stem cells in the toxic demyelinating cuprizone model

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Multiple sclerosis (MS) is an inflammatory demyelinating disease of the central nervous system that leads to demyelination and progressive axonal damage and subsequently to loss of neurological functions. Remyelination is the natural repair mechanism but it is often incomplete or fails in MS lesions. To date, therapeutical treatments to enhance remyelination are not available. Recently, the transplantation of exogenous mesenchymal stem cells (MSC) has emerged as a promising tool to enhance repair processes. MSC has been reported to exert beneficial effects on the course of experimental autoimmune encephalomyelitis (EAE), which presents a widely used inflammatory model of MS. Here, we investigated the role of MSC on remyelination using the toxic cuprizone model of demyelination.

To induce demyelination C57BL/6 male mice were treated with cuprizone for up to 5 weeks. At the peak of disease (week 4) single injections containing 1 million human, murine, or canine MSC were applied into the corpus callosum via a stereotactic procedure. Mouse brains were investigated using histological and immunohistochemical methods.

Our results show that MSC did not exert any effects on cuprizone induced de- and remyelination. Immunohistochemical analyses revealed no changes in myelination and glial reactions including oligodendrocytes, microglia and astrocytes.

In conclusion, local application of MSC was not able to be identified as a new strategy to enhance remyelination in mice in our model of toxic induced demyelination.

T14-22B

The P2X7 receptor is involved in normal re-myelination following sciatic nerve injury

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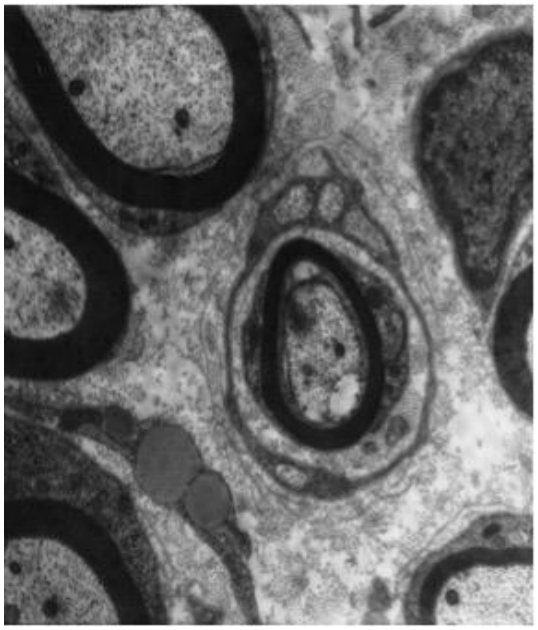
P2X7 is an ionotropic receptor for the purinergic transmitter ATP, and has roles in cell death and inflammation. Our recently published evidence suggests that P2X7 regulates Schwann cell development in peripheral nerves, and by extension contributes to normal myelination. Expanding on this data, we confirm that an additional strain of P2X7 knockout mouse (Pfizer) exhibits the same changes in myelin protein levels as the previously examined strain (Glaxo). In an effort to determine whether P2X7 also controls Schwann cell re-differentiation after injury, we investigated a cut-and-repair sciatic nerve injury model in P2X7 knockout and control mice. Immunohistochemistry revealed no difference in regenerative distance at 1 week post-injury, with regeneration complete in both knockout and control mice by 2 weeks. However, real-time qPCR analysis of sciatic nerve homogenates revealed differences in myelin-related gene expression at 4 and 8 weeks post-injury. Similar analysis of gene expression for markers of neuronal death and inflammation is also being performed in dorsal root ganglia (DRG) neurons belonging to injured nerves at 4 and 8 weeks post-injury. Morphological analyses, using light and transmission electron microscopy, revealed no differences in the number of myelinated axons when comparing knockout and control nerves at 4 weeks post-injury. However, the presence of abnormal myelination was noted in knockout mice, with non-myelinating Schwann cells surrounding myelinated fibres in several instances (figure 1). As such, our data suggest that the P2X7 receptor is not only involved in normal Schwann cell development, but also plays a role in Schwann cell function and re-myelination after sciatic nerve injury. This presents the P2X7 receptor as a potential novel target for treatments of demyelinating neuropathies and peripheral nerve injuries.

Image



T14-23B

Olfactory ensheathing cells overexpressing prostacyclin synthase improves functional restoration after transplantation to transected rat spinal cord

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Olfactory ensheathing cells (OEC), specialized glia that ensheathe bundles of olfactory axons, have been reported as favorable substrate for axonal regeneration. Recent evidence has suggested that OEC graft into injured spinal cord may facilitate axonal regeneration. In an attempt to improve the growth-promoting properties of OEC, the possibility of *ex vivo* adenoviral (Ad) gene transfer of prostacyclin synthase (PGIS) to OEC was examined. Prostacyclin (PGI_2) is known as a vasodilator and cytoprotective agent against various stresses. Overexpression of PGIS in OEC selectively enhanced prostacyclin synthesis. AdPGIS- transduced OEC were further mixed with fibrin glue and transplanted into the completely transected thoracic spinal cord at level 8. Behavior tests (BBB scores) were conducted every other week. By 10 weeks post-surgery, significant functional recovery in hind limb usage occurred in OEC or AdPGIS-infected OEC transplanted rats compared with transected Controls, transplanted with fibrin glue or with culture medium alone. AdPGIS-OEC transplanted rats showed significantly higher BBB score than that of OEC-transplanted rats. Axonal regeneration will be assessed after 12 weeks post-surgery using (1) immunohisto chemistry in the caudal spinal cord to identify whether axons had past the transected site, (2) anterograde tracing of WGA-HRP from sensory motor cortex.

T14-24B

RNA nanoparticles for targeted delivery of siRNAs against reactive astroglial cells - an *in vitro* study

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Question: Due to the temporospatial complexity of spinal cord injury (SCI), treatments reducing neurodegeneration whilst enhancing plasticity, axonal regrowth and functional recovery are still lacking. Reactive astrocytes play a key regulatory role and are marked by upregulation of glial fibrillary acidic protein (GFAP) and vimentin. During the process of astrogliosis astrocytes migrate, undergo hypertrophy, regulate inflammation and remodel the injury niche. Pro-inflammatory factors released by activated astrocytes contribute to secondary degeneration, while hypertrophy and remodelling causes glial scar formation, inhibiting plasticity and regeneration. Preventing GFAP/vimentin upregulation has been shown to reduce scarring and enhance functional recovery. The aim of this study was to develop a drug-delivery system using RNA nanotechnology that could deliver siRNAs to reactive astrocytes to prevent GFAP/vimentin overexpression. An *in vitro* model for astrocyte reactivity was adapted and validated in order to optimize the composition of the nanoparticles (NPs) and to study their efficacy and off-target effects.

Methods: RNA NPs were manufactured by *in vitro* transcription and macromolecular self-assembly. For the *in vitro* model of astrocyte reactivity neural progenitor cells (NPCs) were differentiated into astrocytes and activated with lipopolysaccharide (LPS) and interferon gamma (INFγ). RT-qPCR, ELISA, Western blot, and immunofluorescence were used to validate the model and to study the efficacy and off-target effects of the NPs.

Results: NPC-derived astrocytes showed significant levels of GFAP expression and a stellate morphology after 15 days *in vitro.* LPS/INFγ induced activation of signal transducer and activator of transcription 3 (STAT3) and upregulation of major histocompatibility complex class II (CIITA), nitric oxide synthase 2 (NOS2), interleukin 6 (IL-6), and tumour necrosis factor (TNF). NPs significantly knock down GFAP and vimentin in resting and activated astrocytes at concentrations as low as 0.5 nM. NPs were nontoxic and nonimmunogenic. Knockdown of GFAP and vimentin did not prevent release of IL-6 and TNF by activated astrocytes.

Conclusions: Low concentrations of specifically designed NPs could significantly knock down GFAP and vimentin in NPC-derived resting or LPS/INFγ-activated astrocytes *in vitro*. Although they were not able to prevent the release of IL-6 and TNF, these NPs might prove valuable in reducing hypertrophy, migration and scarring *in vivo*.

T14-25B

Astrocytes enhance the dopaminergic differentiation of stem cells and promote brain repair through bFGF

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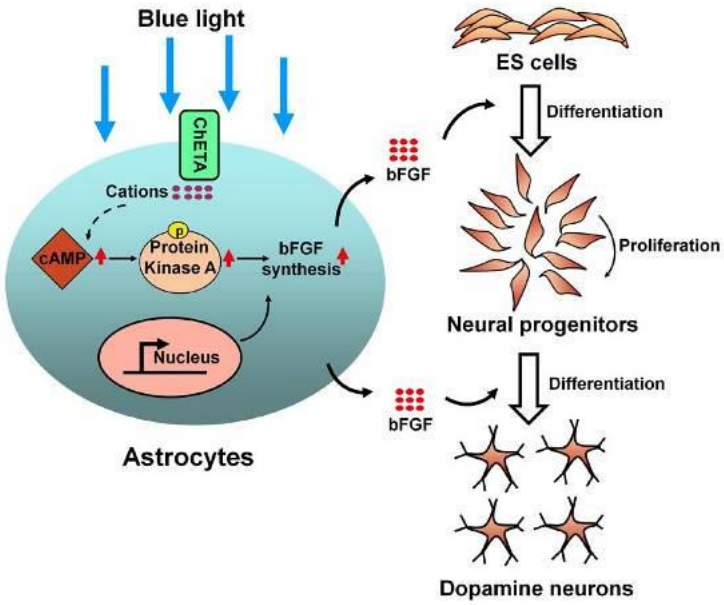
Astrocytes provide neuroprotective effects against degeneration of dopaminergic (DA) neurons and play a fundamental role in DA differentiation of stem cells. Here we show that light illumination of astrocytes expressing engineered channelrhodopsin variant (ChETA) can remarkably enhance the release of basic fibroblast growth factor (bFGF) and significantly promote the DA differentiation of human embryonic stem cells (hESCs). Light activation of transplanted astrocytes in the substantia nigra (SN) also upregulates bFGF levelsin vivo and promotes the regenerative effects of co-transplanted stem cells. Specific light activation of endogenous astrocytes could also upregulate bFGF levels in the SN,enhances the DA differentiation of transplanted stem cells and promotes brain repair in a mouse model of Parkinson's disease (PD). Our study indicates that astrocyte-derived bFGF is required for regulation of DA differentiation of the stem cells and may provide a strategy targeting astrocytes for treatment of PD.

Image



T14-26B

Increasing AMPA signalling to improve myelin repair

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The myelin sheath around axons is essential for normal brain function. There are devastating consequences when myelin is lost in diseases such as multiple sclerosis (MS) making it crucial to understand how to enhance myelin repair. During development myelinating oligodendrocytes are generated from oligodendrocyte precursor cells (OPCs) in a process that can be regulated by neuronal activity. OPCs persist in the adult CNS as a self-renewing population of stem cells. They can differentiate to carry out myelin repair in disease, but remyelination often fails, leaving axons vulnerable to atrophy and making the enhancement of remyelination an important therapeutic aim.

We have recently found that OPCs in the white matter receive synaptic input from neuronal axons and respond to glutamate via AMPA receptors. Demyelinated axons in white matter lesions release glutamate and synapse onto recruited OPCs during myelin repair. Blocking this signalling, e.g. with the AMPA receptor blocker NBQX, prevents OPCs from differentiating and thus blocks remyelination. We are thus investigating if increasing the axon-OPC communication via AMPA receptor agonists can improve myelin repair.

We tested the AMPA receptor activators tianeptine, piracetam, S18986 and CX546. White matter OPCs were patched in acute mouse brain slices and AMPA evoked currents were compared to currents evoked in the presence of the drug. Tianeptine and piracetam - though potent AMPA signalling enhancers in neurons - failed to exert the same effect in OPCs. S18986 is a positive allosteric modulator of AMPA receptors and a cognitive enhancer in rodents and preincubation of brain slices in 100 μM S18986 increases AMPA receptor mediated currents in OPCs by a factor of 7.5. CX546 is an ampakine which binds allosterically to AMPA receptors and slows down the receptor deactivation. Preincubation in 250 μM CX546 increases the AMPA evoked currents in OPCs by a factor of 10.4.

These results show that the AMPA evoked current in OPCs can be enhanced. We are now testing the effect of S18986 and of CX546 on myelination in co-cultures of dorsal root ganglion neurons and OPCs and on remyelination *in vivo* using the ethidium bromide lesion model in rats to evaluate their efficacy as agents to promote myelin repair in white matter disease.

T14-27B

Transcriptional regulation of AMPA-type glutamate receptors in the oligodendrocyte lineage

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AMPA-type glutamate receptors (AMPAR) regulate the development of oligodendrocytes (OL), and mediate damage to these cells and their myelin during excitotoxic injury. The subunit composition of OL AMPAR endows them with a high degree of calcium permeability. In particular, OL express high levels of AMPAR subunits GluR3 and GluR4, whose assembly into AMPAR generates calcium permeable receptors. Over activation of calcium-dependent signalling through these receptors is well placed to contribute to pathological conditions that injure OL and myelin in pre-term infants, and following traumatic injury and stroke. Therefore, a clearer understanding of the transcriptional mechanisms regulating the expression of calcium-permeable AMPAR subunits in OL has the potential to define new pathways for the protection of OL and myelin. We lack knowledge of the transcriptional mechanisms regulating AMPAR subunit expression in the OL lineage. Therefore, we initiated an omics-based search to identify regulators of OL AMPAR. GluR4 is highly enriched in both mature and immature OL, thus we focussed our initial work on Gria4, the gene for this subunit. Bioinformatic analysis of the Gria4 transcriptional network identified a number of transcription factors with the potential to influence Gria4 promoter activity. We are studying the influence of these transcription factors on Gria4 expression at two levels. First, ChIP assays, and luciferase reporter experiments, are being performed to confirm physical and functional interactions between these transcription factors and their binding sites on the Gria4 promoter. Second, the expression of these transcription factors, and that of their target, GluR4, is being examined in an *in vitro* model of excitotoxic injury. This work promises to identify novel targets whose regulation may provide protection to OL and myelin during excitotoxic injury.

Poster topic 15
Transmitter receptors, ion channels and gap junctions

T15-01A

Changes in glial glutamate transporters protein levels in TMEV model of viral-induced epilepsy

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Questions: Viral encephalitis is often associated with seizures and increased risk of developing chronic epilepsy. Intracerebral (i.c) infections of Daniel (DA) strain of Theiler's murine encephalomyelitis virus (TMEV) in susceptible strains of mice cause acute encephalitis followed by a chronic inflammatory demyelinating disease with persistence of the virus (Sato et al., *Pathophysiology*. 18(1): 31-41, 2011). Recently it has been shown that C57BL/6 (B6) mice are resistant to TMEV-induced demyelination and, after developing an acute disease characterized by tonic-clonic seizures, are able to clear the virus within 2 weeks post-infection (p.i.). Interestingly, TMEV infection chronically alters seizure susceptibility and a significant proportion of mice develop spontaneous seizures, establishing the TMEV model in B6 mice as a valid model of viral-induced epilepsy (Figure 1). Astrocytes seem to play a key role in demyelination resistance because, compared to other strains of mice, B6 display an enhanced astrocytic anti-viral response that might contribute to their ability to clear TMEV (Carpentier et al., *Virology*. 375 (1): 24-36, 2008). Nevertheless the mechanisms involved in acute seizures and epilepsy development have not been elucidated yet within this model.

Methods: Increased excitation due to altered glutamate transporters levels has been described in epilepsy (Meldrum et al., *Epilepsy Res.* 36: 189-204, 1999). The aim of our work was to unravel the involvement of glial glutamate transporters in the TMEV model of viral-induced epilepsy. Therefore, protein expression levels of GLAST and GLT1, two glutamate reuptake transporters, and xCT, the functional subunit of cystine/glutamate antiporter or system x_c^- which is the main source of extracellular glutamate in mouse hippocampus, were investigated by Western Blotting. Cortical (CRX), hippocampal (HP) and cerebellar (CER) samples were collected during the acute phase (5 days p.i.), the clearance phase (14 days p.i.) and the chronic phase (2 months p.i.) of B6 mice infected with DA strain of TMEV and compared with their mock-infected littermates.

Results: Our findings (Table 1) show changes in glial glutamate transporters expression that may be involved in seizure generation and development of subsequent epilepsy.

Conclusions: Further research is needed to elucidate whether increased levels of system x_c^- together with changes in glutamate re-uptake carriers lead to a rise in extracellular glutamate levels and hyperexcitability in viral-induced epilepsy.

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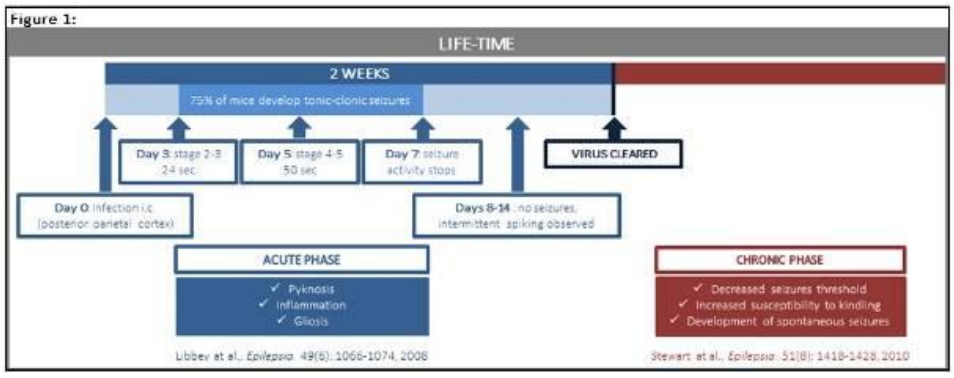

Table

Table 1:											
5days	GLAST	GLT1	xCT	14days	GLAST	GLT1	xCT	2months	GLAST	GLT1	xCT
CRX	↑	↑	↑	CRX	=	↓	=	CRX	↑	=	↑
HP	=	↑	=	HP	=	=	=	HP	=	=	=
CER	=	=	=	CER	=	=	=	CER	=	=	=

T15-02A

Regulation from cytosolic alkalosis by reversed sodium-bicarbonate cotransporter NBCe1 in mouse cortical astrocytes

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The electrogenic sodium-bicarbonate cotransporter (NBCe1, *SLC4 A4*) is a major acid/base transporter in glial cells, particularly in astrocytes and known to operate with a stoichiometry of $1Na^+$:$2HCO_3^-$ (Theparambil et al., 2014). The equilibrium potential of NBCe1 (E_{NBC} -70 to -80 mV) is close to the resting membrane potential of astrocytes. Therefore, depending upon the ion distribution and membrane potential of astrocytes, NBCe1 can function as an acid extruder and acid loader by mediating inwardly or outwardly-directed bicarbonate transport, respectively. In the present study we have investigated the contribution of NBCe1-mediated, outwardly-directed sodium bicarbonate cotransport to the regulation of H^+_i from an acute alkalosis in primary cultured mouse cortical astrocytes of wild-type and NBCe1-KO mouse. The changes in intracellular H^+ and Na^+ during alkalosis were monitored with a confocal microscope (LSM-510) by employing pH- and Na^+-sensitive fluorescent probes, BCECF-AM and ANG-2 AM, respectively. The rate of rise of H^+_i and concomitant acid/base flux rate, as well as the rate of fall of Na^+_i during regulation from alkalosis, were substantially reduced in NBCe1-KO astrocytes as compared to WT astrocytes. This suggests that extrusion of sodium and bicarbonate via outwardly-directed NBCe1 is the major acid loading process, which regulate H^+_i from an acute alkalosis, in our experiments induced by the removal of either 5%CO_2/26 mM HCO_3^- or 40 mM butyrate. Removing chloride had only a minor effect on the recovery from alkalosis, whereas inhibiting CA activity reduced the rate of recovery from alkalosis by up to 40%. Since cells are closely packed in the brain within narrow extracellular spaces, we postulate that the extrusion of sodium-bicarbonate by reversed NBCe1 from astrocytes, supported by CAII activity, may not only play a major role in acid-loading in astrocytes, but also for modulating extracellular pH and H^+ buffering, and hence neuronal activity.

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Reference

Theparambil, S.M. Ruminot, I., Schneider, H.-P., Shull G.E. & Deitmer, J.W. (2014) J. Neurosci. 34:1148-1157.

T15-03A

HCO3--independent pH regulation in astrocytes in situ is dominated by V-ATPase

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BACKGROUND: Studies using cultured astrocytes indicate that the Na-H-exchanger (NHE) is the principal HCO3--independent H+-extrusion protein in the CNS, coupling H+ extrusion to the inward Na+ gradient. Astrocytes also express a V-type H-ATPase that can be blocked by bafilomycin, although the involvement of this pump in pH maintenance and recovery following an acid load is limited in cultured cells. The mechanisms of HCO3--independent intracellular pH (pHi) regulation were examined in in situ fibrous astrocytes within isolated neonatal rat optic nerve (RON) and in cultured cortical astrocytes. RESULTS: In agreement with previous reports, resting pHi in cultured astrocytes was 6.82 ±0.06 and inhibition of the V-ATPase H+ pump by Cl- removal or via the selective inhibitor bafilomycin had only a small effect upon resting pHi and recovery following an acid load. In contrast, resting pHi in RON astrocytes was 7.10 ±0.04, significantly less acid than that in cultured cells, and responded to inhibition of V-ATPase with profound acidification to the 6.3-6.5 range. Fluorescent immuno-staining and immuno-gold labelling confirmed the presence V-ATPase in the cell membrane of RON astrocyte processes and somata. Using ammonia pulse-recovery, pHi recovery in RON astrocyte was achieved largely via V-ATPase with sodium-proton exchange (NHE) playing a minor role. CONCLUSION: The findings indicate that astrocytes in a whole-mount preparation such as the optic nerve rely to a greater degree upon V-ATPase for HCO3--independent pHi regulation than do cultured astrocytes, with important functional consequences.

T15-04A

Connexin channel inhibitor promotes the anti-hyperalgesic effect of amitriptyline in sciatic nerve-ligated rats

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Antidepressants, often prescribed as first line treatment of neuropathic pain, have a limited efficacy and are endowed with poorly tolerated side effects. Because recent studies pointed out the implication of astroglial connexins in neuropathic pain as well as antidepressant-induced changes in connexin 43 (Cx43)-gap junctions, we investigated whether (i) the tricyclic antidepressant amitriptyline could modulate this connexin and (ii) Cx43 inhibition could influence amitriptyline effect.

Rat cortical astrocytic cultures expressing Cx43 and treated by amitriptyline and THN01, a Cx43-based channel inhibitor, were used to assess astroglial coupling with the scrape loading method. Those drugs were then used in vivo in male Sprague-Dawley rats with unilateral chronic constriction injury (CCI) to the sciatic nerve. Two weeks after surgery, rats having developed mechanical hyperalgesia in the nerve lesioned paw were treated for a further two weeks with either amitriptyline (12 mg/kg/day, through a s.c. implanted osmotic minipump), THN01 (0.5 mg/kg i.p. twice daily), the combination of both drugs or their vehicle. The Randall-Selitto's test was then applied to assess time-course changes in mechanical hyperalgesia. At the end of treatments, ipsilateral dorsal root ganglia and dorsal spinal cord at L4-L6 level were dissected out for real-time RT-qPCR quantification of mRNAs encoding markers of CCI-induced neuroinflammation. Amitriptyline levels were also quantified by HPLC in rats' serum and brain.

We found that both amitriptyline and THN01 inhibited, in a synergic way, Cx43-mediated astrocytic coupling. THN01, inactive on its own on nocifensive responses, significantly enhanced amitriptyline-induced reduction of mechanical hyperalgesia. Amitriptyline pharmacokinetics did not apparently differ whether or not rats had been co-treated with THN01, and CCI-induced overexpression of IL-6, IL-1ß, ATF3 and OX-42 transcripts in ganglia/spinal cord was not significantly affected by treatments.

These data showed that the connexin inhibitor THN01 enhanced amitriptyline antihyperalgesic effect in the rat CCI model, and that both drugs acted synergistically to inhibit Cx-mediated astrocytic coupling. Unchanged overexpression of neuroinflammatory markers suggests that the antihyperalgesic effect of combined amitriptyline+THN01 treatment involved mechanisms downstream of these processes. Inhibition of Cx-based channel functions in astroglia could represent a promising approach toward improving neuropathic pain therapy.

T15-05A

Gating of aquaporin 4 - phosphorylation versus protonation

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Aquaporin 4 (AQP4) is the predominant water channel in the mammalian brain and is mainly expressed in the perivascular glial endfeet at the brain-blood interface. AQP4 is an important entry site for water during brain edema formation and regulation of AQP4 is therefore of therapeutic interest. Aquaporin-mediated water permeability has been proposed to be altered by a gating mechanism within the protein upon phosphorylation of Ser^{111}. By a range of complementary experimental strategies, we failed to obtain evidence in favor of phosphorylation-dependent gating of AQP4 via Ser^{111}, whether in cultured astrocytes or upon heterologous expression of AQP4 in oocytes (with or without an intact Ser^{111}). AQP4 phosphorylated at Ser^{111} was not detected in vivo and experimental mimicking of a phosphorylated Ser^{111}, whether with a mutational strategy or via molecular dynamics simulations, recorded no difference in the water permeability of AQP4. Alternatively, while extracellular pH changes did not affect AQP4 water permeability, intracellular acidification led to increased AQP4-mediated water permeability. Both experimental data and molecular dynamics pointed to pH-dependent protonation of His^{95}, located in the pore region facing the cytoplasm, as the gating mechanism underlying the pore-opening.Thus, phosphorylation-dependent gating of AQP4 at Ser111 might not, as earlier proposed, act as the molecular switch for astrocytic cell swelling or brain edema formation whereascellular acidification increases the osmotic water permeability of AQP4-expressing cells.

T15-06A

Prenatal exposure to inflammatory conditions increases hemichannel opening and activation of astrocytes in the offspring: repercussion on neuronal survival

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Several epidemiological studies indicate that children from mothers exposed to infections during gestation, have an increased risk to develop neurological disorders including schizophrenia, autism and cerebral palsy. However, whether astrocytes and their crosstalk with neurons participate in the above mentioned brain pathologies remain to be elucidated. Our aim was to address if paracrine signaling mediated by hemichannels could be affected in astrocytes from the offspring of LPS-exposed dams during pregnancy. Ethidium uptake experiments showed that prenatal LPS-exposure increases the activity of astroglial connexin 43 (Cx43) and pannexin 1 (Panx1) hemichannels in the offspring. Moreover, induction of hemichannel opening by prenatal LPS exposure depended on intracellular Ca^{2+} levels, cytokine production? and the activation of the p38 MAP kinase/iNOS pathway. Biochemical assays and Fura-2AM/DAF-FM time-lapse fluorescence images revealed that astrocytes from the offspring of LPS-exposed dams display increased spontaneous Ca^{2+} dynamics and NO production, whereas iNOS levels and release of IL-1β/TNF-α were also increased. Interestingly, we found that prenatal LPS exposure enhanced the release of ATP through astroglial hemichannels in the offspring, resulting in increased neuronal death induced by the activation of neuronal $P2X_7$ receptors and Panx1 hemichannels. Thus, altogether this evidence suggest that astroglial hemichannel opening induced by prenatal LPS exposure depended on the inflammatory activation profile and the activation pattern of astrocytes. The understanding of the mechanism underlying astrocyte-neuron crosstalk could contribute to the development of new strategies to ameliorate the brain abnormalities induced in the offspring by prenatal inflammation.

T15-07A

Expression of functional ionotropic glutamate and GABA receptors in astrocytes of the ventrobasal thalamus

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Astrocytes may express transmitter receptors, which allow them to sense and to respond to neuronal activity. By now, astrocytic neurotransmitter receptor expression was characterised only in a few brain regions. Here we provide a detailed analysis of AMPA and $GABA_A$ receptors in astrocytes of the mouse ventrobasal thalamus. Receptor currents were investigated using the patch clamp-technique combined with single cell RT-PCR to explore receptor subunit expression.

To improve voltage-clamp control and avoid indirect effects, freshly isolated cells were employed using juvenile hGFAP/EGFP mice. Application of the AMPA receptor agonist, kainate, supplemented with cyclothiazide (CTZ), which modulates AMPA receptors, lead to a 6-fold potentiation of the kainate-induced responses. I/V relations of the kainate/CTZ-induced receptor responses were linear with a reversal potential close to 0 mV and were almost completely blocked by the AMPA receptor blocker GYKI 53655. Two sub-populations of astrocytes were found, expressing or lacking AMPA receptors. AMPA receptor-bearing astrocytes displayed a lower density of inwardly rectifying K^+ (Kir) currents than those cells lacking the receptors. The relative Ca^{2+}-permeability of the receptors, P_{Ca}/P_K, was determined using Na^+-free, high Ca^{2+} (50 mM) bath solutions and amounted to $P_{Ca}/P_K = 0.15$. Thus, AMPA receptors of juvenile thalamic astrocytes show a low Ca^{2+}-permeability. Single-cell transcript analysis of AMPA receptors revealed a prevalent expression of GluA2 and GluA3 subunits.

$GABA_A$ receptors currents were found in each cell. The cells were exposed to muscimol which produced desensitizing inward currents that were sensitive to bicuculline. Most frequently, expression of the subunits α2, α5, β1, γ1 and γ3 was observed.

Our findings add to the emerging concept of functional heterogeneity between astrocytes within and across brain regions.

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T15-08A

Dye coupling between cells from subventricular zone neurospheres and glia

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The postnatal subventricular zone lining the lateral ventricles contains neural progenitor cells (NPCs) that generate new olfactory bulb interneurons. Communication via gap junctions between NPC-derived neuroblasts and between neuroblasts and niche astrocytes modulates neuroblast proliferation and migration towards the olfactory bulb. Subventricular zone NPCs can be isolated and expanded *in vitro* in the form of neurospheres. Neurosphere-derived NPCs have been widely used for transplantation purposes in different types of brain lesions. We have previously reported that NPCs establish gap junctions with host glial cells when they are implanted after mechanical injury (Talaverón et al., Glia 2014). In order to analyze whether NPC-glial cell gap junctions are functional we performed dye coupling experiments in co-cultures of subventricular zone neurosphere-derived cells and primary astrocytes or microglia. Neurosphere-derived cells expressed mRNA for the hemichannel/gap junction channel proteins connexin 43 (Cx43), Cx45, Cx26 and pannexin 1. Hemichannel activity was also observed in neurosphere cells in time-lapse measurements of ethidium bromide uptake. Dye coupling experiments revealed that cell-cell coupling occurred among cells in neurospheres (incidence of coupling: 100%; index of coupling: 3.0 ± 0.3). A strong NPC-astrocyte cell coupling was also detected (incidence of coupling: 91.0 ± 4.7%; index of coupling: 2.4 ± 0.3) between neurosphere-derived cells

and astrocytes maintained in co-culture. Heterocellular coupling between neurosphere-derived cells and microglia was also evident in co-culture experiments (incidence of coupling: 71.9 ± 6.7%; index of coupling: 2.1 ± 0.4). Altogether, these results propose the existence of functional cell-cell coupling among cells within postnatal subventricular zone neurospheres. In addition, they demonstrate that neurosphere-derived cells can establish gap junctional communication with astrocytes or microglia. Therefore, gap junctional communication with host glial cells might be involved in the integration, survival and functionality of NPCs after implantation in the lesioned brain.

T15-09A

The HYS-32-enhanced Cx43 stability at plasma membrane is caveolae-dependent

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HYS-32 is an analogue of combretastatin A-4 (CA-4) previously shown to increase caveolin-3 (Cav3) expression and upregulate connexin43 (Cx43) protein levels and gap junction intercellular communication in rat primary astrocytes. This study aims to elucidate the effects of HYS-32 on Cav3 and Cx43 expression and the signaling pathway involved. Confocal microscopy with double immunofluorescence staining of Cx43 and Cav3 demonstrated that HYS-32 induces an increased colocalization of Cx43 and Cav3 at cell-cell contact sites in astrocytes. In a time course study, HYS-32 upregulated Cx43 protein levels after treatment for 6-24 h, whereas the expression of Cav3 increased at 6 h, reached its peak at 18 h, and backed to normal at 24 h. The expression of Cav3 mRNA was upregulated after HYS-32 treatment for 3-6 h and rapidly declined from 12-18 h. HYS-32 treatment for 24 h also increased phospho-PKC and phospho-ERK levels. The HYS-32-induced increase in Cav3 and Cx43 levels were prevented by co-treatment of HYS-32 with PKC inhibitor Gö6976 or ERK inhibitor PD98059. Gö6976 also inhibited the upregulated effect of HYS-32 on phospho-ERK expression. By discontinuous sucrose-gradient centrifugation, HYS-32 induced a significant shift of Cx43 into the lipid raft buoyant fractions as the buoyant/heavy ratio of Cx43 greatly increased following HYS-32 treatment. By immunoprecipitation, an increased association of Cav-3 and Cx43 was also detected in HYS-32-treated astrocytes in both total protein lysates and Triton X-100 insoluble fractions. Exposure of methyl-β-cyclodextrin (MβCD), a caveolae (caveolin-rich membrane domains) disruptor, disturbed the HYS-32-increased colocalization of Cx43 with Cav3. Further, co-treatment with HYS-32 and cycloheximide delayed Cx43 turnover, whereas MβCD abolished the HYS-32-induced deceleration of Cx43 turnover. Together these findings suggest that HYS-32 upregulates Cav3 and Cx43 via the PKC-ERK signaling pathway and that the HYS-32-enhanced stabilization of Cx43 at plasma membrane is caveolae-dependent.

T15-10A

Functional characterization of astrocytes within the ventral midbrain

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Astrocytes are ubiquitous in the brain, but vary dramatically across brain regions in their morphology, gene expression, and likely function. Very little is known about astrocytes in the substantia nigra and ventral tegmental area - two midbrain dopaminergic areas implicated in multiple adaptive and pathological behaviors, including reward and substance use disorders. A number of published reports provide indirect evidence for astrocyte dysfunction in addiction, but direct, mechanistic studies of how astrocytes are involved in its development and expression are lacking. In order to study how astrocyte function changes in pathological states, we first set out to define the baseline properties of these cells and determine the effects of dopamine signaling on their basal activity. We performed whole-cell patch-clamp recordings and immunohistochemistry in adult Aldh1L1 eGFP mice, as well as calcium imaging in acute slices expressing GCaMP6m. Under basal conditions, astrocytes in the midbrain express significantly lower levels of traditional glial markers than in their hippocampal counterparts.

They exhibit passive membrane conductances similar to those described in other regions, but the relative contribution of potassium channel subtypes to this conductance differs from what was observed in the hippocampus. In addition to large spontaneous somatic calcium fluctuations, bath application of dopamine receptor agonists produced decreases and increases in cytosolic calcium levels within individual astrocytes. Collectively, these results demonstrate that ventral midbrain astrocytes have unique molecular and electrophysiological profiles and can be indirectly modulated by dopamine receptor signaling. These data provide a foundation for future experiments looking at whether astrocyte activity is influenced by and/or influences drug-induced changes in the dopaminergic system.

T15-11A

Regulation of BDNF mRNA expression in astrocytes by catecholamines

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The brain-derived neurotrophic factor (BDNF) gene has a complex structure with several promoters to allow regulated tissue-specific expression. It was revealed already in early studies of the 1990s that BDNF expression is induced by different stimuli in neurons and astrocytes - by glutamate in neurons and by catecholamines in astrocytes (Zafra et al., 1990, Zafra et al., 1992). During the past decades, BDNF expression in neurons has been thoroughly investigated, whereas astrocytic BDNF expression has caught much less attention, mostly due to low expression levels of BDNF in cultured astrocytes. However, recent RNAseq data of different cell types in the mouse cortex showed that astrocytes in vivo expressed BDNF mRNA at levels comparable to those of neurons (Zhang et al., 2014), suggesting that astrocytic BDNF may have a significant contribution to the roles of this neurotrophin in the brain. Catecholamines are known to upregulate BDNF mRNA expression (Zafra et al., 1992) and BDNF protein secretion (Miklič et al., 2004, Jurič et al,. 2008, Ohta et al., 2003). Here we revisit regulation of BDNF mRNA in cultured astrocytes, focusing on transcriptional regulation of alternative BDNF promoters. Our results show that BDNF promoter VI is robustly activated by dopamine and norepinephrine via cAMP signaling and promoter IV is induced to a lesser extent. BDNF mRNAs expressed under the control of promoters I and II are virtually undetectable in cultured astrocytes. Preliminary results from promoter assays suggest the existence of a remote cis-regulatory element that controls induction of BDNF promoters IV and VI by catecholamines.

T15-12A

AMPA receptor signalling in oligodendrocyte development

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Myelin in the CNS is produced by oligodendrocytes (OLs), which are themselves produced by oligodendrocyte precursors (OLPs, also known as NG2 cells). NG2 glia are generated during embryonic development and continue to proliferate and give rise to new OLs throughout life. They express AMPA receptors (AMPAR) and receive AMPAR-mediated glutamatergic synaptic input from neurons. It has long been known that glutamate can influence OLP proliferation and/or differentiation in vitro, but the role of AMPAR signalling in OL development in vivo has not been addressed in detail. We examined the expression of AMPAR subunits in cells of the OL lineage by in situ hybridization and immunolabelling. We found that GluA2, GluA3 and GluA4 subunits are expressed in OLPs in the white matter (corpus callosum) whereas GluA1 is absent. In P14 brain slices, conditional homozygous knock-out of GluA2 in the OL lineage using Sox10-Cre, in mice with GluA3 globally knocked out, resulted in a more than 50% reduction in kainate-evoked current at -60mV in OLPs, and induced strong inward rectification, compared to mice heterozygous or wild type (wt) for GluA2 and lacking GluA3. The reduction in kainate-evoked current was accompanied by a >70% reduction in ruthenium red-evoked EPSC frequency, with no change of amplitude. The densities of OLPs and their differentiated OL progeny in vivo were also reduced relative to GluA3 KO controls. There was no

detectable difference in the proliferation rate of OLPs at P3 and P14. These results suggest that AMPARmediated signalling plays a role in regulating OL development in vivo.

T15-01B

Short-term modulation of astrocyte plasma membrane extensions by GPCRs

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Question: In the last decades astrocytes have emerged as important synergic cells in brain function, having roles in trophic support, synaptic activity and vascular modulation. Most of these functions are mediated by astrocyte plasma membrane (PM) extensions, expressing both aquaporin-4 and K^+ inward rectifying (K_{ir}) channels. Since astrocytes might be exposed to different stimuli and several pathways are activated by G protein coupled receptors (GPCR), we asked whether PM extensions and their function might be altered over time upon metabotropic receptor activation/deactivation.

Methods: Isolated rat hippocampal astrocytes were used for live cell imaging experiments and whole-cell patch-clamp recordings. For imaging, cells were stained with the PM dye CellMask Orange and monitored for 20 min after drug treatments. In patch-clamp experiments, astrocyte K_{ir} current was recorded applying voltage-steps (-180 mV to 60 mV) following 0 mV holding potential.

Results: We found that PM extensions are modulated in number and length by GPCR, mainly by Gq-mediated pathway. To elicit Gq activation we selected endogenous purinergic receptor $P2Y_1$. In western blot analysis, we observed an increased expression of $P2Y_1$ receptor in cultured cells up to three weeks. In live cell imaging experiments, we validated functional $P2Y_1$ receptor by monitoring calcium flux with Fluo-4 indicator. Activation of $P2Y_1$ by the selective agonist 2-methylthioadenosine diphosphate (2MeSADP, 100 mM) showed increased number of PM extensions that was prevented by pre-treatment with selective $P2Y_1$ antagonist, MRS 2179 (1 mM). To test the effective contribution of Gq-mediated pathway, we performed live cell imaging experiments in cells treated with phospholipase C inhibitor U73122 (3 mM). We observed a dramatic decrease of PM extensions in length and number. Based on this observation we hypothesized that reduction of extensions will alter K^+ channel activity. K_{ir} current was recorded in basal condition and after U73122 application. A dramatic increase in current through K^+ channels at negative potentials (-180 mV to -100 mV) was observed. Cells were monitored for 10 min after U73122 application and such effect was achieved after 3 min treatment. At positive potentials (20 mV to 60 mV), K^+ current was reduced over time up to 10 min.

Conclusions: These data suggest that astrocytes extensions are dynamically modulated by GPCR and that astrocytes K^+ buffering function might be altered by impaired Gq-mediated pathways.

T15-02B

Unravelling the mechanisms causing astrocytic uncoupling in the epileptic hippocampus

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Astrocytes are connected to each other by gap junctions composed mainly of Connexin43 (Cx43) and Connexin30 (Cx30). These networks have been proposed to counteract epileptic activity by facilitating removal of extracellular K^+ and glutamate. Recently, we got evidence that 1) astrocytic coupling is lacking in the sclerotic hippocampus, both in human and experimental epilepsy, and 2) astrocytic uncoupling is a very early event in the process of epileptogensis, indicating that it is causative and not merely a consequence of the disorder. An experimental model of epilepsy involving intracortical injection of kainate in mouse was employed to investigate underlying mechanisms. We chose 3 time points after kainate injection: 4 hr - after this time point apoptotic neurons begin to appear, 5 days - latent phase ends and spontaneous seizures start, and 3 months - astrocytic coupling is completely abolished, recapitulating findings in human epileptic hippocampus associated with sclerosis. First we checked the expression of Cx43 protein in our mouse model and revealed that it was not reduced at

any of the time points studied despite of reduced coupling. This indicates a role of post translational modification of connexins in uncoupling. Indeed we found that phosphorylated forms of Cx43 were elevated throughout all three time points. Surprisingly, at 3 months after kainate injection, expression of phosphorylated and non-phosphorylated Cx43 was enhanced. Given the prominent role played by phosphorylation in the function of Cx43, currently we are trying to assess putative phosphorylation sites with mass spectrometry. Protein levels of Cx30, which in the hippocampus has a smaller contribution to overall coupling (approximately 20%), is reduced at 4 hr after kainate injection. However, Cx30 expression is enhanced after 5 days and after 3 months. Understanding of how these changes in protein expression relate to the observed loss of coupling is currently under investigation. We also study the subcellular distribution and phosphorylation of Cx43 in hippocampal specimens of epileptic patients. Our goal is to identify new pharmacological targets to prevent or rescue astrocytic uncoupling.

T15-03B

Inducible astrocyte specific Kir4.1 knockout mice exhibit a blunted ventilatory response to CO_2

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The brain regulates breathing in response to changes in tissue CO_2/H^+ via a process termed central chemoreception. An area on the ventral medullary surface of the brainstem called the retrotrapezoid nucleus (RTN) has been identified as a key locus for chemoreception and central control of respiration. Neurons in this region can sense CO_2/H^+ (i.e. are chemosensitive) in part by inhibition of TASK-2 channels. RTN astrocytes are also chemosensitive and CO_2-evoked release of the gliotransmitter ATP most likely occurs via connexin 26 hemichannels. This purinergic component appears to gain up RTN neuron chemosensitive responses by approximately 30%. However, RTN astrocytes also express a H^+-sensitive current mediated by a Kir4.1-like conductance. The contribution of this pH sensitive current in RTN astrocytes to the central respiratory drive has yet to be determined. Here we generate an inducible astrocyte specific Kir4.1 knockout (Kir4.1 cKO) using GFAP-CreERT2 and Kir4.1 floxed mouse lines. We confirmed immunohistochemically that expression of Kir4.1 in GFAP positive cells was reduced in the hippocampus, cerebellum and RTN. Furthermore, whole-cell voltage clamp recordings of RTN astrocytes in brain slices from Kir4.1 cKO mice showed the absence of a Kir4.1-like current. Preliminary cell attached current-clamp recordings from RTN chemoreceptor neurons in slices from Kir4.1 cKO mice appear to show a reduction in the purinergic component of their CO_2/H^+ response, suggesting deletion of Kir4.1 from astrocytes may disrupt the ability of these cells to respond normally to changes in CO_2/H^+. To determine whether Kir4.1 cKO mice exhibit altered respiratory drive, we measured respiratory activity in awake mice by whole-body plethysmography during graded increases in CO_2. We found that Kir4.1 cKO animals breathe normally under control conditions, but hypoventilate in 100% O_2 and show a reduced tidal volume response to CO_2 compared to litter mate controls. These results suggest that Kir4.1 channels in astrocytes contribute to the central drive to breathe.

T15-04B

Subcellular distribution and trafficking of astroglial receptors monitored with super-resolution microscopy

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Astrocytes play an active role in shaping and maintaining neuronal circuits. The cells constantly sense the surrounding milieu. They regulate neuronal activity, synaptic transmission and plasticity through secretion and clearance of neurotransmitters. Neuronal activity induces astrocytic morphology and protein expression changes, which in turn shapes synaptic activity and neuronal excitability; thus predicting a constant interaction between neurons and glial cells. So called perisynaptic astrocytic processes respond especially to synaptic activity with induced motility, altering the coverage of synaptic structures by astrocytes.

We employ super-resolution microscopy techniques such as PALM (photoactivated localization microscopy) and dSTORM (direct stochastical optical reconstruction microscopy) to visualize the position and dynamics of metabotropic receptors and neurotransmitter transporters in living and fixed astrocytes. These methods rely on the sequential activation, imaging and bleaching of a sparse subset of fluorescent molecules, either photoactivatable proteins or photoswitchable fluorophores. Thus, images can be obtained with sub-diffraction resolution by localizing individual activated molecules in each frame. Using dSTORM, we were able to localize cytoskeletal proteins and clusters of receptors in astrocytic membranes in fixed cells and brain slices. Moreover, through multi-color staining, the positional relationship between synapses and astroglial receptors and transporters can be assessed. Using PALM, clusters of receptors can be imaged and the number of molecules can be counted. Additionally, the position of each photo-converted molecule can be tracked for a short period of time before photobleaching which allows the generation of trajectories of several well separated molecules over a given period of time. We are now trying to image the recruitment of receptors and transporters from intracellular vesicular stores and the diffusion within the plasmamembrane in cultured astroglia.

T15-05B

Glutamine synthetase stability is by regulated by g-aminobutyric type B receptors

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GABABRs are heterodimeric G-protein coupled receptors that are composed of R1/R2 subunits. To date, they have been characterized in neurons as the principal mediators of sustained inhibitory signaling, however their roles in astrocyte physiology are ill defined. Recently our laboratory showed that the cytoplasmic tail of the GABABR2 subunit binds directly to the astrocytic protein glutamine synthetase (GS) and that this interaction increases the steady state expression levels of GS (Huyghe et al., 2014). Here we have examined the significance of this interaction for GS stability under metabolic stress. Our results suggest GS binding to GABABRs increases during oxidative stress to limit GS degradation. Given the critical role that GS plays in several neurological diseases, we propose that astrocytic GABABRs enhance GS stability under pathological conditions to ensure the integrity of the glutamate-glutamine cycle.

T15-06B

Functional expression of GABAρ in astrocytes from neostriatum

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The neostriatum plays a central role in motor coordination where nerve cells operate neuronal inhibition through GABAergic transmission. The neostriatum expresses a wide range of $GABA_A$ mRNAs, including those of GABAρ subunits and scattered information has suggested the expression of GABAρ in glial cells from this area; thus the aim of this study was to determine whether GABAρ subunits are expressed in astrocytes and to characterize the functional properties of the GABA-responses. Whole-cell recordings showed GABA-evoked currents (119 ± 22 pA; n = 12) that were bicuculline-sensitive, although a bicuculline-resistant current was observed (17± 9 pA; n = 6). This current was blocked when TPMPA, a selective GABAρ antagonist, was combined with bicuculline (5 ± 3 pA; n = 7; completely abolished in 5 out of 7 cells), suggesting that a fraction of the GABA-evoked current was generated by GABAρ subunits. In support of this, TPMPA alone reduced GABA-evoked currents by 53% (from 122 ± 26 pA to 58 ± 10 pA; n=3). RT-PCR revealed the expression of GABAρ3 subunit through development while immunohistofluorescence disclosed its expression in astrocytes. We conclude that astrocytes from neostriatum present a GABAρ component in addition to the classic $GABA_A$ receptors.

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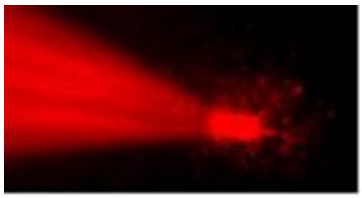
Image



T15-07B

Astrocyte swelling in response to neural activation: role of cotransporters

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Among the many active roles playedby astrocytes, we can mention K+ clearance and glutamate uptake from the extracellular space (ECS) during neuronal activation. The mechanisms underlying these specific functions are accompanied, for osmotic reasons, by transmembrane water movements, which challenge the astrocyte volume constancy. Consequently, taking into account cell volume homeostasis is an essential condition for survival, in particular in the brain where consequences of volume deregulation can be dramatic (oedema, ischemic and hemorrhagic strokes, etc.).Itis likely that cell volume regulatory mechanism are also involved in the processesofion clearance and neurotransmitter uptake.

Practically, to grasp thesemultifaceted astrocyte processes, we have developed amultimodal approach, which combines both quantitative phase digital holographic microscopy (QP-DHM) and epifluorescent imaging. Thisapproach thus providesthe original ability to simultaneously monitor both cell volume and transmembrane water movements as well as the intracellular concentrations of the specific ionic species including $[Na^+]_i$ and $[K^+]_i$.Preliminary results obtained from such multimodal measurements performed on primary cultures of mouse astrocytes have confirmed that:

1) Increased extracellular K+levels causes astrocyte swelling through mechanisms involving the activation of sodium-potassium-chloride cotransporter NKCC1.

2) Glutamate applicationsof 200µM during 2 minutesinduce astrocyte swelling through the activation of GLAST glutamate transporters.

However, these multimodal measurements have permitted to stress that water influx are temporally offset by the GLAST-mediated [Na+]i rise and continuesafter washout of glutamate, suggesting that the glutamate mediated net water influx must depend upon another mechanism that still remains to beclarified. In addition, astrocyte cell volume regulation after glutamate applicationstronglydepends on extracellular [K+].

T15-08B

Astrocytic pH-regulation in cell culture of mice

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Cellular pH homeostasis is a complex and highly ramified occurrence. Homeostasis of pH depends on a variety of regulating processes, like H^+ or HCO_3^--influx, -efflux, buffering and extra-/intracellular carbonic anhydrase (CA) activity, last of them developed to increasingly demand in recent years.

Many astrocytic transporters are involved in primary or secondary pH regulation, like the sodium hydrogen exchanger (NHE 1) or electrogenic sodium bicarbonate cotransporter (NBCe1), or causing intracellular pH shifts (pH_i) after activation, as glutamate transporters and receptors. Transporters are essential for taking up released glutamate or aspartate (Asp), receptors mediate astrocytic 'response processes' after neuronal activity, both fulfilling astrocytic part of mental activity.

By using cell culture of hippocampal astrocytes, we focused on intracellular CAII. We applied wide field imaging and used the AM-ester of pH-sensitive dye 2´,7´-biscarboxyethyl-5,6-carboxyfluorescein (BCECF) to monitor astrocytic pH changes. Cells were measured with a tuneable laser connected to Till software, enabling us to measure ratiometrically. In a new approach to define subcellular varieties in pH-regulation, soma and branches were analyzed by using a separate calibration for each of them. First of all buffer capacities (β) were analyzed and compared by application and withdrawal of a weak acid. The reaction of astrocytes to glutamate and Asp as well as lactate was tested and analyzed regarding the two compartments (soma and extensions). Still under investigation are the common acid/base regulating proteins, like NHE1 or NBCe1, and their contribution to D-Asp- or Glu-induced pH_i-changes. We use common inhibitors to silence their function in the cellular interplay.

First results show significant differences between soma and extensions. Buffer capacity is at least twice as large in extremities, depending on the kind of calibration we use. Rate of proton changes ($\Delta H^+/t$ [nmol/min]) is several times smaller, respectively. Imitation of transmitter release cause faster and larger H^+ disturbances in branches compared to somata.

In the future we want to test, if pH_i-changes due to glutamate or D-Asp are solely due to transporter activity or either rely on receptor activation (AMPA) by using AMPA-specific blocker. Furthermore we are looking forward investigating astrocytic pH_i changes in the thin processes of astrocytic culture from CAII-KO-mouse strain, to evaluate the role of carbonic anhydrase II in regulating subcellular pH-homeostasis.

Image

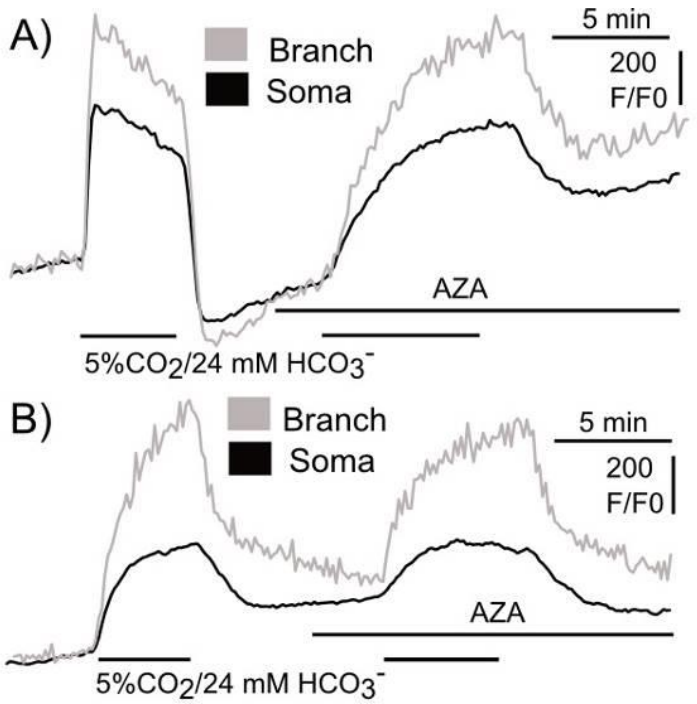

Fig 1: Original traces of cultured astrocytes in HEPES loaded with BCECF, stimulated by a weak acid, CAII blocked by 100 µm AZA
A) acidic range, starting from 7.0 (branch) and 6.6 (soma)
B) alkaline range, starting from 7.7 (branch) and 7.5 (soma)

T15-09B

Molecular mechanisms underlying nodal protein assembly prior to myelination in the CNS

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The fast and reliable propagation of action potentials along myelinated fibers relies on the clustering of voltage-gated sodium channels (Na_v) at the nodes of Ranvier. While mechanisms underlying nodal formation in the peripheral nervous system are well understood, their formation in the central nervous system (CNS) remains to be fully elucidated. Recent work by our group has shown that pre-nodes, i.e. evenly spaced aggregates of Na_v, the cytoskeletal scaffolding protein ankyrin-G (AnkG) and the cell-adhesion molecule neurofascin-186 (Nfasc186), form along GABAergic axons in the absence of myelination *in vitro* and also in the developing hippocampus (Freeman *et al*., 2015). Importantly, single-axon electrophysiological recordings comparing GABAergic unmyelinated axons with and without pre-nodes revealed that there is a 1.5-fold increase in conduction velocity along axons with these clusters, representing a novel means for accelerated axonal propagation in the absence of myelin. Nevertheless, pre-nodal formation requires oligodendroglial soluble factors, highlighting the importance of neuronal-glial interactions in this process.

Although the ultimate clustering of various nodal components requires the contribution of oligodendrocytes, intrinsic neuronal properties also govern the compartmentalization of these proteins to the axonal plasma membrane. To gain further insight into the intrinsic dynamics of CNS nodal clustering, we are now using time-lapse imaging of fluorescently tagged nodal markers along axons in hippocampal mixed neuron glia cultures. Further studies are currently being performed in order to understand the transport dynamics of these varying nodal markers and determine which particular motor proteins perturb axonal transport of these key nodal components prior to pre-nodal formation.

T15-10B

Mechanosensitive ion channel, Piezo1, is expressed in myelinated regions of the rat brain

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Piezo1, also known as FAM38A, is a non-selective calcium (Ca^{2+})-permeable cation channel that produces rapidly inactivating currents in response to membrane stretch (Coste et al. 2010). Mechanically-activated (MA) ion channels can translate external physical stimuli to intracellular biochemical signals in a process termed *mechanotransduction*. Piezo1 plays important roles in tissues subject to regular and extensive mechanical stretch, including the lungs, bladder and vasculature (McHugh et al. 2012; Li et al. 2014; Miyamoto et al. 2014). It is also important for pain perception since capsaicin-mediated TRPV1 channel activation can inhibit rapidly-adapting Piezo1 MA currents and this is dependent on extracellular calcium influx (Borbiro et al. 2015).

At present, very little is known about the function of Piezo1 in the central nervous system (CNS). In the healthy brain, Piezo1 is expressed by neurons but absent in astrocytes. In patients suffering from Alzheimer's disease, however, Piezo1 mRNA is downreguated in neurons and upregulated in astrocytes surrounding amyloid plaques (Satoh et al. 2006). Therefore, Piezo1 was previously known as 'membrane protein induced by beta-amyloid treatment' (i.e. Mib). However, the specific brain regions and neuronal cell types that express Piezo1 under normal physiological conditions have not yet been characterised.

Here, we performed an immunofluorescence study to characterise Piezo1 protein expression and localisation in the young and adult rat brain. We found that myelinated neurons, in particular, express high levels of Piezo1 mechanosensitive ion channels. Piezo1 is expressed in white matter-rich areas including the corpus callosum, cingulum, habenula, anterior commissure, fimbria, internal capsule and optic tract. Piezo1 is also highly expressed in the arbor vitae region of the cerebellar lobules. Piezo1 is expressed on neurons before extensive postnatal myelination takes place, although its expression

increases significantly in white matter brain regions by six weeks of age. Cerebellar Purkinje neurons also express high levels of Piezo1 in adult rats.

In future studies, we aim to elucidate why neurons in myelinated areas of the brain express high levels of the mechanosensitive ion channel, Piezo1. Currently we are investigating if Piezo1 plays a novel role in developmental myelination in the cerebellum.

T15-11B

The role of L-type calcium channels subtypes Cav1.2 and Cav1.3 in NG2 glia

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Oligodendrocyte precursor cells (OPCs) are an abundant class of glial cells in the CNS characterized by expression of the chondroitin sulfate proteoglycan NG2. Therefore, OPCs are also designated as NG2 glia. Voltage-gated Ca^{2+} channels (VGCCs), which have been detected on oligodendrocyte lineage cells *in vitro* and *in vivo*, are suggested to play a fundamental role at early stages of OPC maturation. Using acute brain slices prepared from developing and mature NG2-EYFP mice, we found that in different brain regions during development membrane depolarization elicited inward currents in all NG2 glia which were abolished by the L-type Ca^{2+} channel blocker nimodipine, and, indeed, recently mRNAs encoding the L-type Ca^{2+} channel a-subunits Cav1.2 and Cav1.3 were detected in hippocampal NG2 glia.

To study the functional role of L-type Ca^{2+} channels in NG2 glia at different developmental stages, we used the inducible Cre/lox system (CreERT2) and crossbred NG2-CreERT2 knock-in to respective Cav1.2 and Cav1.3 mice. Deletion of the Ca^{2+} channel genes was induced by intraperitoneal injection of tamoxifen in one and three week old mice.

Three weeks after tamoxifen injection, whole-cell patch-clamp recordings showed significantly reduced peak currents of L-type Ca^{2+} channels when elicited by a voltage step to 0 mV. Other whole-cell currents such as K^+ currents were not affected in Cav1.2/1.3 deficient NG2 glia. We are currently investigating how the removal of Cav1.2/1.3 alters proliferation, differentiation and migration of NG2 glia. A particular focus lies on the analysis of synaptic signaling generated by Cav1.2/1.3 deficient NG2 glia.

T15-12B

Anatomical analysis of mutant mice expressing type-I cannabinoid receptors in astrocytes of the hippocampus

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The type-1 cannabinoid (CB_1) receptor is probably the most widely expressed G protein-coupled receptor in the brain where it mediates the effects of (endo)cannabinoids on neuronal transmission, plasticity and other functions. Anatomically, CB_1 is expressed at low levels in certain cell types and subcellular compartments, as well as mitochondria of the brain, that have often been misinterpreted as background staining. Hence, the deletion of a gene in cells expressing very low levels of the protein implies the risk to miss the complete pattern of expression. Therefore; to identify low levels of expression, “rescue” strategies are needed. However, to establish whether genetic re-expression maintains normal CB_1 expression in specific cell types, the distribution pattern has to be evaluated in these mutants in detail.

The aim of this study was to analyze the subcellular distribution of CB_1 in CA1 hippocampal astrocytes of conditional "CB_1 rescue mice" which re-express CB_1 only in astrocytes (GFAP-*CB_1*-RS mice) and transgenic mice carrying GFP under the control of the GFAP promoter (GFP-GFAP mice). Specific CB_1, GFAP and GFP antibodies combined with a preembedding immunogold and immunoperoxidase methods for electron microscopy were applied to hippocampal sections of those mutants, as well as of *CB_1*-WT and *CB_1*-KO mice used as controls.

The results showed that 36.61% ± 3.03% of astrocytic sections were CB_1 immunopositive in GFAP-*CB_1*-RS. No significant differences were observed with the CB_1 immunopositive astrocytic elements in *CB_1*-WT mice (42.17% ± 3.20%). Just residual unspecific particles were detected in astrocytes in *CB_1*-KO (2.33% ± 1.01%) and GFAP-*CB_1*-KO mice (4.32% ± 1.27%). In GFP-GFAP mice, 60.54% ± 2.55% of the GFP immunoreactive astrocytic processes were CB_1 positive (significant difference compared to *CB_1*-WT, **; $p < 0.05$).

To summarize, the proportion of CB_1 immunopositive astrocytic processes in the CA1 hippocampus of *CB_1*-WT is maintained in GFAP-*CB_1*-RS mice. The results demonstrate the great potential of these transgenic mice to study CB_1 in brain astrocytes where the localization of this receptor is sparse. In addition, more CB_1 positive astrocytic processes were observed in GFP-GFAP mice, suggesting that better astrocyte detection can be achieved in this mutant. Hence the expression of CB_1 on astrocytic processes might be actually higher than what was previously expected.

T15-13B

Generation of conditional knockout mouse lines for opioid receptors in microglia

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Aim of investigation: Recently, some evidence for activated microglia being key-players in chronic pain development and persistence has been described. Moreover both microglia and astrocytes have been proposed as critical actors in opiate-induced hyperalgesia and tolerance. The opioid receptors are G protein-coupled receptors and can be found under three different types, mu, delta and kappa, all of them inducing analgesia. Delta opioid receptors (DOR) are of prime interest as potential therapeutic targets for chronic pain and mood-related pathologies, besides the mu-opioid receptor that represents the major target of clinically used opiates.

Methods: In the present study, we focused on the role of microglial DOR, by generating new conditional knockout mouse lines with a specific deletion of DOR in microglia. We crossed the previously described DOR-floxed mice (Gaveriaux-Ruff et al. (2011) Pain 152:1238-1248) with CX3CR1-Cre (Goldmann et al. (2013) Nat Neurosci 16:1618-1626) or LysM-Cre (Clausen et al. (1999) Transgenic Res 8:265-277) microglia deleter mice. We characterized this new mouse lines by molecular and cellular approaches, followed by behavioral pain tests. In addition, we study the impact of the DOR deletion in microglia on inflammatory pain, following Complete Freund's Adjuvant (CFA) injection into tail, as a model for inflammation-induced pain. Floxed and full DOR knockout animals were used as controls.

Results and conclusions: Basal nociceptive thresholds were unchanged in DOR conditional knockout mice as compared to controls. Local tail inflammatory pain developed over several days following CFA in the DOR mutant and control mice.

Taken together, these preliminary results provide informations on microglial DOR implication in pain perception, and suggest that glial DOR are of prime interest in studying the implication of this receptor in chronic pain.

Poster topic 16
Trophic factors

T16-01A

Changes in expression and localisation of Sphingosine 1-Phosphate Receptor-1 ($S1P_1R$) in the young and middle-age rat brain

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Sphingosine 1-phosphate (S1P) is a bioactive lipid generated from sphingosine through the action of sphingosine kinases (SphK1 and SphK2). S1P regulates many important cellular functions such as angiogenesis, neural development, cell migration, cell survival and lymphocyte chemotaxis. It achieves these pleiotropic effects through activation of a family of five G protein-coupled receptors (GPCRs) named $S1P_1R$ to $S1P_5R$. S1P receptors show cell type-specific expression patterns which underpin its wide-ranging functions in the cardiovascular, immune, and central nervous systems (CNS).

This study focuses on the S1P receptor subtype-1 ($S1P_1R$). Here, we investigate how $S1P_1R$ expression changes in the rat brain from youth to adulthood. $S1P_1R$ is involved in various key CNS functions during development, including vascular maturity and microglial migration. The $S1P_1R$ is coupled to the Gi/o alpha subunit and thus inhibits adenylyl cyclase and reduces cAMP production in cells. Activation of $S1P_1R$ also results in a rise in intracellular calcium levels which can activate many downstream signalling cascades leading to cytoskeletal rearrangements.

Using immunofluorescence and image analysis techniques, we analysed $S1P_1R$ expression and regional localisation in P9, P37 and P450 day old rat brains. At 9 days old, $S1P_1R$ is highly expressed in most cortical layers with lower levels in the auditory cortex. It is also highly expressed in the non-neuronal cell layers of the hippocampus and cerebellum. At 37 days old, $S1P_1R$ expression appears to decrease in the cortex and increase in the hippocampus. This pattern of expression is generally maintained in 450 day old rat brains with the highest expression measured in the CA1 stratum radiatum and CA1 stratum oriens regions of the hippocampus. $S1P_1R$ is also highly expressed in the granular and molecular layers of the cerebellum at 450 days old. $S1P_1R$ expression appears predominantly non-neuronal as it does not co-localise with neurofilament H staining.

The redistribution of the $S1P_1R$ in the rat brain from neonatal developmental stages through to adulthood may represent key changes in its role in CNS functioning. Future work will focus on determining the role of $S1P_1R$ in the hippocampal CA1 region of aged rats.

T16-01B

Neuroprotection and reduction of astroglial reaction by human embryonic stem cell engrafting following spinal cord ventral root avulsion

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Ventral root avulsion (VRA) is a proximal lesion to motoneurons in the spinal cord. Such injury triggers strong glial reaction that contributes to further neuronal loss as well as to the formation of glial scar. Neurotrophic factor and stem cell treatments have been proposed as promising approaches to overcome the degenerative effects of VRA, by supporting neuronal survival and axonal regrowth. Thus, the present study investigated the neuroprotective effects of human embryonic stem cells (hESC), modified to overexpress FGF-2 in an inducible manner, on motoneurons subjected to VRA. Cells were applied to the injury site by the use of a fibrin scaffold (FS). For this, female Lewis rats were randomly distributed in the following groups: **1-** VRA + FS (no-hESC-control); **2-** VRA + FS + doxycycline (DX); **3-** VRA + FS + recombinant FGF2 (trophic factor control); **4-** VRA + FS + hESC (FGF2 secretion OFF) and **5-** VRA + FS +hESC + DX (FGF2 overexpression ON). Transgene overexpression was induced by doxycycline intake mixed with pelleted food. Two weeks after lesion,

neuronal survival was evaluated by Nissl staining. Glial reaction and synaptic network preservation were evaluated by immunohistochemistry. The results showed that treatment with hESC induced to overexpress FGF2 increased neuronal survival by 20%. Immunohistochemistry labelling with synaptophysin (a pan synaptic input marker) indicated preservation of overall input network to motoneurons (**1:** 45,20%; **2:** 41,33%; **3:** 58,06%; **4:** 52,16% **5:** 74,3%; integrated density of pixels - ratio ipsi/contralateral; $p < 0.0001$). In addition, a significant decrease of glial fibrillary acidic protein (GFAP - astroglial marker) labeling, following engraftment of FGF2 overexpressing hESC, was found (**1:** 178,7%; **2:** 173,1%; **3:** 154,2%; **4:** 154,9%; **5:** 125,5%; $p<0.0001$). No detectable effects of stem cell treatment were depicted on microglial reaction, investigated with Iba-1 (Ionized calcium binding adaptor molecule 1) antibodies (**1:** 278,5%; **2:** 319,2%; **3:** 244,3%; **4:** 273,4% **5:** 247,8%). The results herein suggest that overexpression of FGF2 by engrafted hESC leads to neuroprotective effects following proximal lesions to the spinal cord. Importantly, such transgenic cell therapy not only enhanced neuronal survival, but also decreased astroglial reaction, which may in turn facilitate axonal regrowth, if ventral root repair is carried out. Overall, the present data may lead to promising regenerative studies, aiming at improving motor recovery after brachial and lumbosacral plexus lesions.

T16-02B

Age related loss of oligodendrocyte metabolic support

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In the CNS oligodendrocytes are well established as being involved in myelination of axons and providing saltatory conduction of action potentials. Oligodendrocytes are generated from oligodendrocyte progenitor cells (OPCs), which mildly proliferate and differentiate under normal physiological conditions and more so upon oligodendrocyte injury. Besides their role in myelination, our laboratory and others have found that oligodendrocytes are important mediators of metabolic support to ensheated neurons through their expression of monocarboxylate transporter 1 (MCT1). MCT1 is involved in shuttling metabolic substrates like lactate, pyruvate and ketone bodies together with hydrogen ions across plasma membranes. In our laboratory it has been shown that local lentiviral mediated loss of MCT1 expression in oligodendrocytes leads to axonal degeneration both in vitro and in vivo. In this study, we will use transgenic mouse tools to further explore the basic biology of MCT1 expression in the CNS during aging under normal physiological conditions. We found that MCT1 mRNA expression remained unaffected during aging both using MCT1-tdTomato mRNA reporter mice as well as qPCR of different spinal cord and brain gray and white matter regions. In order to gain further insight into the role of MCT1 in oligodendrocyte maturation, and to examine whether adult OPCs still have the capacity to generate MCT1-expressing oligodendrocytes, we performed in vivo genetic fate tracing of OPCs using PDGFRaCreER mice crossbred with YFP reporter mice and MCT1-tdTomato reporter mice. We found that up to the age of 20 months, the ability of newly oligodendrocytes to turn on MCT1 mRNA expression was unaffected by aging. Interestingly, and in contrast as what is seen for MCT1 mRNA, MCT1 protein expression was significantly reduced in both brain and spinal cord at both the ages of 12 months and even further at 20 months, suggesting posttranslational modifications affected MCT1 protein expression during rodent aging. Whether this loss of support is also found in human oligodendroglia is unknown. The loss of MCT1 expression was concomitant with a loss of other oligodendrocyte markers like CNPase. Using MCT1 conditional knockout mice, we are currently trying to exploring the how this loss of oligodendrocyte MCT1 expression affects normal neuronal homeostasis.

Poster topic 17
Tumours

T17-01A

Glioma-initiating cells upregulate IL-6 secretion in microglia/brain macrophages via Toll-like receptor 4 signaling

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Peripheral macrophages and resident microglia constitute the dominant glioma-infiltrating cells. The tumor induces an immunosuppressive and tumor supportive phenotype in these glioma associated microglia/brain macrophages (GAMs). A subpopulation of glioma cells has stem cell properties such as self-renewal, pluripotency and act as glioma-initiating cells (GICs). In the present study we explored the interaction between GICs and GAMs. Using CD133 as a marker of stemness, we either enriched for or depleted the mouse glioma cell line GL261 of GICs by FACS or used the sphere-forming capacity to identify GICs. After stimulation of primary cultured microglia with conditioned medium from CD133 enriched GL261 glioma cells we observed an upregulation in microglial IL-6 secretion while medium from CD133 depleted gliomas did not trigger this release. This upregulation was selective for IL-6 as compared to a battery of other cytokines (e. g. TNF-a, IL-2 or IL-4). This upregulation depended on Toll-like receptor (TLR) 4, a pattern recognition receptor which can trigger pro-inflammatory cytokines release. The effect was abolished in the $TLR4^{-/-}$ mice, but not in other strains deficient for other TLRs. The implantation of GL261-EGFP cells into the brains of $IL\text{-}6^{-/-}$ mice resulted in significantly smaller tumors as compared to wild type control mice. Only 100 $CD133^{+}$ GICs were sufficient to form tumors of a comparable size formed by 10000 $CD133^{-}$ GL261 cells. In $IL\text{-}6^{-/-}$ mice, only tumors formed by $CD133^{+}$ cells were smaller when compared to control mice. Our results show that GICs, but not the bulk glioma cells initiate microglial IL-6 secretion via MyD88-TLR4 signaling and IL-6 regulates glioma growth by influencing GICs. Using human glioma tissue we could confirm the finding that microglia are the major producers of IL-6.

T17-02A

Inhibition of glioma progression by a newly discovered CD38 inhibitor

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Glioma, the most common cancer of the central nervous system, has very poor prognosis and no effective treatment. It has been shown that activated microglia/macrophages in the glioma tumor microenvironment support progression. Hence, inhibition of the supporting effect of these cells may constitute a useful therapeutic approach. Recently, using a syngeneic mouse glioma progression model, we showed that the ectoenzyme CD38 regulated microglia activation and, moreover, that the loss of CD38 from the tumor microenvironment attenuated glioma progression and prolonged the life span of the tumor-bearing mice. These studies, which employed wild-type (WT) and *Cd38*$^{-/-}$ C57BL/6J mice, suggest that inhibition of CD38 in glioma microenvironment may be used as a new therapeutic approach to treat glioma. The present study tested this hypothesis. Initially, we found that the natural anthranoid, rhein, and its highly water soluble tri-potassium salt form (K-rhein) are inhibitors of CD38 enzymatic (NAD^{+} glycohydrolase) activity (IC_{50} = 1.24 and 0.84 μM respectively, for recombinant mouse CD38). Treatment of WT, but not *Cd38*$^{-/-}$ microglia with rhein and K-rhein inhibited microglia

activation features known to be regulated by CD38 (LPS/IFNγ-induced activation induced cell death and -NO production). Furthermore, nasal administration of K-rhein into wild-type, but not *Cd38*$^{-/-}$ C57BL/6J, mice intracranially injected with GL261 cells substantially and significantly inhibited glioma progression. Hence, these results serve as a proof of concept demonstrating that targeting CD38 at the tumor microenvironment by small molecule inhibitors of CD38 *e.g.*, K-rhein, may serve as a useful therapeutic approach to treat glioma

T17-03A

Adoptive M1/M2 modulation of microglia as an immunotherapeutic strategy against glioma

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Glioblastoma multiforme(GBM) is a highly aggressive primary brain tumor. It has an exceptionally poor patient prognosis despite current multimodal therapy treatment, which includes the outright or partial resection of the tumor bulk followed by radiation and adjuvant chemotherapy. The immune mechanisms in GBM enclose the dynamic response of immune cellular players, including glioma associated microglia/macrophages (GAMs). This immune surveillance which is present in the tumor micro-environment and in the extracellular matrix of the CNS is shifted towards an immunosuppressive state by glioma cells. The GBM immunosuppressive capabilities and mechanisms can arise from a combination of factors that involves GAMs modulation through GAMs-glioma signaling. The recognition of the immunomodulatory properties of cancer cells while promoting suitable tumor micro-environment, underlines the need for appropriate models for studying GBM biology and for predicting its response to immune surveillance. In the present work we aim to characterize the glioma response upon an adoptive immunotherapeutic strategy based on M1/M2 modulation of glioma in an in-vitro-three dimensional (3-D) model that we developed. The model consists of multicellular spheroids embedded in a collagen matrix. Immune modulation by GBM cells and their capacity to invade in absence or presence of M1-like or untreated microglia was studied using spheroids of murine SMA-560 astrocytoma cells mixed with murine primary microglia. Our data show that collagen-implanted M1-like microglia may inhibit growth of the mixed tumor-microglia spheroids at early time points, but are later polarised by glioma cells towards a M2-like profile. However, this M2-like polarization is successfully inhibited when interleukin-6 (IL-6) is neutralized. We are currently evaluating our working hypothesis on the role of a IL-6/STAT3 signaling pathway as an importantinducer of tumor cell invasion via enzymatic activity of MMP-9. Our present findings indicate that the 3-D gel-based *in vitro* model is a promising system for studying GBM biology and microenvironment signaling. As such, it represents an attractive model that might be used for the assessment of different treatments and the prediction of their impact.

T17-04A

A cell division cycle 7-related protein kinase inhibitor suppresses glioblastoma cell growth *in vitro*

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Eukaryotic DNA replication is an evolutionarily conserved mechanism that is subject to stringent spatial and temporal regulation. Cell division cycle 7-related protein kinase (CDC7)/activator of S phase kinase (ASK) complex regulates the initiation of DNA replication by phosphorylating minichromosome maintenance proteins (MCMs). Chemical inhibitors of the CDC7/ASK complex have been tested in different types of cancer, and exert antiproliferative and apoptotic effects. Glioblastoma is the most aggressive and most common malignant brain tumor in humans. The aggressive nature of the tumor and development of rapid resistance to chemotherapeutic agents limit the efficacy of existing treatment strategies. Here, we aim to test a CDC7 inhibitor, PHA-767491, as a novel chemotherapeutic agent for glioblastoma treatment. PHA-767491 treatment drastically decreased cell viability, suppressed cell proliferation, and triggered apoptosis in different glioblastoma cell lines.

Altogether, our findings suggest that inhibiting CDC7/ASK activity could be a new strategy for glioblastoma therapy.

T17-06A

A promising therapy against human glioblastoma stem cells: cell-penetrating peptides based on the interaction between connexin43 and c-Src

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Astrocytes are the most numerous glial cells and they are widely communicated by gap junctions. The main gap junction channel-forming protein in astrocytes is the connexin43 (Cx43). This protein is downregulated when astrocytes acquire a malignant phenotype and originate brain tumours called gliomas. Cx43 has been described to exert a tumour suppressor effect through the interaction with the oncoprotein c-Src, as our previous work has shown in gliomas. Interestingly, Cx43 is down-regulated in glioma stem cells (GSCs), a subpopulation of cells thought to be responsible for tumor initiation, relapse, and therapeutic resistance in gliomas. Restoring Cx43 levels reverses GSCs phenotype and consequently reduces their tumorigenicity. On this basis, we have designed several cell-penetrating peptides (CPPs) containing different regions of Cx43 involved in c-Src interaction and we have investigated their role in GSC proliferation and migration.

Human G166 GSCs, patient-derived GSCs, C6 rat glioma cells, neurons and astrocytes cultures were treated with CPPs. Cell growth was analyzed by MTT. Migration was studied using wound-healing assays, Time-Lapse live-cell Imaging and Immunocytochemistry.

Our results show that CPPs are internalized and reduce the rate of cell growth. Interestingly, the effects of CPPs are stronger in GSCs compared to other type of cells. TAT sequence did not significantly change the rate of growth. Although several studies show that Cx43 increases cell migration, our findings indicate that the region of Cx43 that interacts with c-Src does not exert this effect. In fact, the CPPs used in this study reduced the rate of GSC migration and locomotion.

In conclusion, our results indicate that c-Src plays an essential role in the effects of Cx43 on GSCs proliferation and migration and suggest these CPPs as promising therapies.

T17-07A

Light-controlled inhibition of malignant glioma by opsin gene transfer

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Glioblastomas are aggressive cancers with low survival rates and poor prognosis because of their highly proliferative and invasive capacity. In the current study, we describe a new optogenetic strategy that selectively inhibits glioma cells through light-controlled membrane depolarization and cell death. Transfer of the engineered opsin ChETA (engineered Channelrhodopsin-2 variant) gene into primary human glioma cells or cell lines decreased cell proliferation and increased mitochondria-dependent apoptosis, upon light stimulation. These optogenetic effects were mediated by membrane depolarization-induced reductions in cyclin expression and mitochondrial transmembrane potential. Importantly, the ChETA gene transfer and light illumination in mice significantly inhibited subcutaneous and intracranial glioma growth and increased the survival of the animals bearing the glioma. These results uncover an unexpected effect of opsin ion channels on glioma cells and offer the opportunity for the first time to treat glioma using a light-controllable optogenetic approach.

T17-08A

The role of Cytoplasmic Polyadenylation Element Binding proteins in the pathogenesis of gliomas

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Polyadenylation of cytoplasmic RNA molecules is one of the posttranscriptional mechanisms that play an essential role in the regulation of gene expression. Cytoplasmic polyadenylation element binding proteins (CPEBs) associate with specific consensus sequences of 3'-untranslated regions of mRNA and regulate translation initiation. In basal conditions, CPEBs lead to transcriptional repression, but following phosphorylation of the CPEBs the translation of transcripts is rapidly induced by cytoplasmic polyadenylation. Abnormal expression of these proteins correlates with certain types of cancer, indicating that this form of regulation is important in the control of cell cycle progression, growth and senescence. So far, little is known about the contribution of translational gene regulation by CPEB proteins to the molecular pathology of gliomas. Preliminary evidence supports a role of CPEBs in malignant transformation and identified them as a target for epigenetic inactivation.

Therefore we decided to determine the methylation status of CPEB genes and to investigate its impact on CPEB proteins expression in gliomas. Methylation of the CPEB1 gene was observed in anaplastic astrocytomas and glioblastomas, whereas CPEB3 methylation appeared mainly in glioblastomas. Weak expression of CPEB1 and CPEB4 proteins was found in the majority of investigated tumors in areas of infiltration and vascular proliferation. On the other hand, expression of CPEB2 and CPEB3 appeared to be the most abundant among all CPEBs. Furthermore we noted altered patterns of immunoreactivity against CPEB1 and CPEB3 proteins. Expression of CPEB1 was decreased while CPEB3 level remained elevated in parallel to the growing grade of glioma malignancy. In summary our data suggest dysregulation of CPEB expression in gliomas. This is particularly relevant to glioblastomas, which are highly invasive and poorly respond to most forms of therapy. However, further functional analyses are needed to determine the impact of CPEBs on posttranscriptional mechanisms controlling mRNA translation and to identity CPEB-dependent molecular pathways in glioma cells.

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T17-09A

GFAP in astrocytic tumors

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The glial fibrillaric acidic protein (GFAP) is an intermediate filament (IF) protein that is highly expressed in astrocytes and is often used as a marker for mature astrocytes as well as a biomarker of brain tumors of astrocytic origin (astrocytoma). These tumors are thought to arise from astrocytes, adult neural stem cells, or glia progenitors. Astrocytoma are subdivided in different grades of malignancy and the most malignant astrocytoma (WHO grade IV) is highly invasive, resistant to current therapies, has a high chance of recurrence, and results in a very poor prognosis for patients. Multiple studies report decreased GFAP expression of high grade compared to low-grade astrocytoma relating to its dedifferentiated state. However, GFAP is alternatively spliced and different isoforms are expressed. Where the most abundantly expressed canonical GFAP isoform, GFAPα, is high expressed in mature astrocytes, the alternatively spliced GFAP isoform, GFAPδ, is highly expressed in human adult neural stem cells. The GFAPδ cell-type specific expression, its different C-terminal protein interacting domain, the importance of the GFAPδ/GFAPα ratio for the integrity of the IF cytoskeleton and for the interaction with the extracellular matrix, all hint towards a GFAPδ isoform specific function. Interestingly, recent studies have found a positive correlation of GFAPδ immunoreactivity with astrocytoma grade, in contrast to the negative correlation found for pan-GFAP. Therefore, we hypothesize that the stoichiometry of the GFAP isoforms might be involved in determining astrocytoma malignancy. Such an involvement has been observed for other IF proteins in non-glioma cancer subtypes. Specifically, the reorganization of IF composition during cell migration is thought to be highly important for tumor cell invasion. Subtle changes in the expression of IF proteins and stoichiometry are thought to modulate the interaction of the cell with its environment, e.g.

protrusion, adhesion, and physical properties, thereby controlling migration. Changes in GFAP alternative splicing could therefore change the interaction of astrocytoma cells with their environment, their migration properties and their invasive capacity. The results of our studies in which we modulate the expression of GFAP isoforms in astrocytoma cell lines shows changes in the expression of genes such as laminins and integrins, proteins involved in extracellular matrix (ECM) composition and ECM-cell interactions that regulate cell migration and tumor invasion. Future studies will involve *in vitro* studies of astrocytoma cells, *ex vivo* studies in a tumor invasion model and the analysis of tumor patient material to determine the function of GFAP isoforms in astrocytoma tumor invasion.

T17-01B

Diffuse low grade gliomas: characterization and development of in vitro model for designing innovative therapeutic approaches

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Diffuse low-grade glioma (DLGG) are slow growing grade II tumors that develop in different functional places in the brain and affect young patients between the second and the fourth decades of life. Although, they are clinically stable over a long period of time, these tumors can ineluctably progress to a higher grade of malignancy leading to anaplasia, which then rapidly compromises patient survival. Most grade II gliomas (around 80%) have a false sense mutation (R132H) in the IDH1 gene (isocitrate dehydrogenase) combined with a 1p19q codeletion, mutations in p53 or ATRX. IDH1 mutations induce major dysregulations of DNA methylation leading to defects in cell differentiation. These tumors, classified as astrocytoma or oligodendroglioma, are probably derived from neural progenitors which remain in the adult brain. Today, one important obstacle preventing the development of new therapies for DLGG is the lack of understanding of the cellular composition of these tumors and the absence of an *in vitro* model due to their low proliferation rate. The first aim of our study is to characterize by immunohistochemistry the different pools of cells causing the heterogeneous profile of the tumor and to understand the different signaling pathways that are activated and/or inhibited, as well as the interactions between the cancerous cells and their environment. The second part is to use different approaches in order to enhance the cell proliferation including cell immortalization via expressing or inhibiting the different signaling pathways that are involved in the cell cycle.

T17-02B

Aquaporin 4 related orthogonal arrays of particles undergo drastic changes in pathological conditions like Astrocytomas WHO-Grade II to IV

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At the healthy blood-brain barrier (BBB) astrocytic endfoot membranes contacting the glial-endothelial basal lamina are full of orthogonal arrays of particles (OAP) containing the main water channel of the brain, aquaporin-4 (AQP4). At the parenchymal sides of astrocytes where the membranes lose their contact to the basallamina, AQP4 and OAPs are only rarely found . This demonstrates that at the healthy BBB astrocytes are polarized cells so that water can flow rapidly and very effectively in both directions from the vessel into the brain and vice versa in order to maintain homeostasis. Under pathologic conditions like in astrocytoma WHO-Grade II to IV, the situation has changed: AQP4 waterchannels are distributed evenly over the whole cell and less OAPs are found but hypoxic clusters of particles are observed. AQP4 has different isoforms, the important of which are AQP4-M23 and -M1. M23-transfected cell cultures reveal OAP lattices and a lot of single OAPs whereas M1-transfected cells only show few and small OAPs. In cell membranes of astrocytomas WHO-Grade II-IV we found single OAPs and hypoxic clusters in freeze fracture analyses. This was the reason for us to examine AQP4-M1 and AQP4-M23 expression using quantitative PCR. The results showed an upregulation of AQP4-M1 confirming the freeze fracture analyses showing only few OAPs. Here we show for the first time that AQP4-M1 is upregulated in glioma cells.

T17-03B

Molecular mechanisms of Notch1-induced pericyte-like transdifferentiation of glioblastoma stem cells

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Glioblastoma Multiforme (GBM, WHO grade IV) is the most aggressive and infiltrative of primary brain tumors. Extensive vascularization in necrotic areas is an important hallmark of GBM, and current therapeutic strategies focus on targeting tumor angiogenesis. GBM origin is still unknown but it was shown that subpopulations of multipotent stem-like cells exist within the tumor, and are responsible for relapse. The Notch signaling pathway is central in the maintenance and proliferation of these cells within the perivascular niche, where GBM stem cells closely interact with endothelial cells. Here, we questioned whether Notch1 pathway activation in such cells could influence the extent of tumoral vascularization and dissemination, using an activated form of the Notch1 receptor (NICD). We observed that although Notch1 receptor was activated, the typical target transcription factors (HES5, HEY1, HEY2) were not or barely expressed in our GBM stem cell cultures. Notch1 activation in these cells was found to alter cell morphology, reduce their growth rate and inhibit their migration. This was accompanied by a transcriptional switch where neural stem cell transcription factor expression was reduced (OLIG2, SOX2) while HEY1/2, KLF9 and SNAI2 transcription factors were upregulated. Microarray and proteomic analyses showed that NICD induced the expression of vascular adhesion proteins (ICAM1, VCAM1), angiogenic cytokines (IL8, PLGF), and angiogenic metalloproteinase MMP9. Remarkably, NICD expression also induced the expression of pericyte markers in vitro (NG2, PDGFRβ and αSMA). When xenotransplanted, Notch1 stimulated cells resulted in poorly-disseminating but highly-vascularized tumors containing round and normalized vessel structures with large lumen. Cells also expressed pericyte markers in vivo and closely associated with host endothelial cells, which confirmed a pericyte-like differentiation of GBM stem cells. Our current project focuses on deciphering the transcriptional mechanisms underlying this phenotypic switch. We are especially interested in how SNAI2 and KLF9 are involved in Notch1 induced dormancy and pericyte-like features, by using in vitro mechanistic approaches as well as studying their function in the context of GBM perivascular niches.

T17-04B

Role of the RNA-binding protein HuR in neurofibromas and malignant peripheral nerve sheath tumour

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Neurofibromatosis type 1 (NF1) is one of the most common genetic disorders among humans, affecting 1:3500 individuals worldwide. The hallmark of NF1 is the development of multiple benign peripheral nerve sheath tumors known as dermal and plexiform neurofibromas. Approximately 10% of plexiform neurofibromas undergo malignant transformation into malignant peripheral nerve sheath tumors (MPNSTs), aggressive and highly metastatic soft tissue sarcomas that are essentially incurable. Schwann cells are the crucial pathogenic cell type in NF1. The RNA-binding protein (RBP) HuR is aberrantly expressed in several types of cancers, in which it regulates the expression of several cancer-related proteins. A strong correlation has been found between HuR expression and advancing stages of malignancy.

We observed that HuR expression was significantly increased in NF and MPNST samples compared to normal nerves by IHC, qPCR and WB analysis, and found a strong correlation between HuR expression and degree of malignancy. RIP-CHIP analysis showed that the number of mRNAs bound to HuR increased as malignancy progresses. Amongst them, several ones with well-defined roles in

oncogenesis were identified. Lentiviral-mediated HuR silencing *in vitro* using MPNST cell lines significantly reduced the expression of these genes, as well as proliferation, migration, colony formation and invasion, and also made these cells more sensitive to apoptotic death by UV irradiation. Injection of control and *HuR*-silenced S462 MPNST cells in athymic nude mice was used to assess the physiological role of HuR *in vivo*. Unlike control cells, we found that the *HuR*-silenced cells were not able to develop tumors. To examine the mechanisms that contribute to the elevated HuR levels observed in MPNSTs, we analyzed the levels of several microRNAs in human samples by qPCR. microRNAs downregulated in MPNST compared to neurofibromas and control samples were used to computationally predict if they associate with HuR. We identified miR-146a and miR-342 as negative regulators of HuR, since their overexpression *in vitro,* in MPNST cell lines, led to a reduction of HuR mRNA and protein levels.

In summary, we found that control of gene expression by HuR could be an important determinant in regulating the oncogenic charateristics of MPNSTs and we propose HuR as a possible therapeutic target for these tumors.

T17-05B

Human monocyte-derived macrophages exposed to glioblastoma cells and tumor-associated microglia/macrophages differ in glutamatergic gene expressions

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Dysregulation of glutamate signaling in the brain is a key factor in the invasion and growth of glioblastoma (GBM), the most aggressive primary brain tumor. Glioblastoma cells secrete high levels of glutamate into the extracellular milieu, triggering neuronal cell death through the over-excitation of glutamate receptors and subsequently providing more space for tumor cells to invade and expand. Tumor-associated microglia/macrophages (TAMs), consisting of both parenchymal microglia and monocytes-derived macrophages (MDMs) recruited from the blood, populate up to one third of the tumor and exhibit an alternative, tumor-supporting phenotype. Their response to the dysregulation of glutamate signaling in a glioblastoma microenvironment has seldom been addressed. Given their capacity to induce expression of glutamate transporters and glutamine synthetase in neuropathological conditions, microglia and macrophages may have a functional role in glutamate signaling. To gain insights into the role of TAMs in a dysregulated glutamate signaling environment, we examined and compared the gene expressions of several glutamatergic markers in TAMs and MDMs by quantitative real-time PCR analysis. The following cell types were analysed: freshly-extracted TAMs from GBM; cultured TAMs extracted from GBM as well as cultured MDMs extracted from the blood of healthy volunteers, which the latter two cell types were later exposed to glioblastoma cell lines or normal astrocytes. While freshly-extracted TAMs, cultured TAMs as well as cultured MDMs exposed to glioblastoma cells all exhibited a similar anti-inflammatory response, the genomic profiles relating to glutamate signaling among these cell types were disparate. We observed an increased expression of glutamate transporters EAAT1 and EAAT2 in both freshly-extracted and cultured TAMs, suggesting that these cells might compensate for the lack of glutamate uptake by glioblastoma cells. In addition, these cells elicit a protective response against cytotoxicity by increasing expressions of GluA2 and xCT. However, these changes were not mimicked by MDMs. The only similarity observed between TAMs and MDMs was the increased glutamine synthetase expression to potentially reduce intracellular glutamate levels and glutamate release. Our data strengthens the concept of functional differences between TAMs and MDMs and demonstrates a need to better understand the role of TAMs during dysregulation of glutamate signaling in the GBM microenvironment.

T17-06B

Transport of branched-chain ketoacids is mediated by monocarboxylate transporters in brain tumor cells

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Our group recently showed that sustained glioblastoma growth requires catabolism of branched-chain amino acids (BCAAs). The first step of this pathway is catalyzed by the enzyme branched-chain amino acid transaminase 1 (BCAT1) which transfers the amino group of valine, leucine and isoleucine to alpha-ketoglutarate, resulting in the production of glutamate and the respective branched-chain ketoacids (BCKAs) which can be further catabolized and enter the TCA cycle. We could also show that a substantial fraction of the glutamate is excreted by the tumor cells, consistent with previous studies that have implicated glutamate excretion in tumor-associated epilepsy and enhanced tumor cell growth and migration. In our own recent work we found that glioblastoma cell lines excrete BCKAs in addition to glutamate. It previously had been shown by others that BCKAs can be transported across the cell membrane by monocarboxylate transporters (MCTs), albeit with lower affinity than their predominant substrates such as lactate.

Here we used different approaches to investigate the involvement of MCTs in the transport of BCKAs across cell membranes.

Using *Xenopus* oocytes as a heterologous expression system we showed that co-expression of BCAT1 and either MCT1 or MCT4 facilitated the excretion of BCKAs from the oocytes. These data indicate that MCTs are sufficient to transport BCKAs across the membranes of living cells. When treating glioblastoma cell lines with the MCT1 inhibitor AR-C155858 at concentrations that did not affect cell proliferation rates, we observed significant reductions of BCKAs excretion, suggesting that MCT1 indeed is involved in BCKAs transport in brain tumor cells. Furthermore, data from *in situ* proximity ligation assay (PLA) suggest that BCAT1 is located in close vicinity to MCT1 in the U87-MG glioblastoma cell line. We hypothesize that this might result in increased local concentrations of BCKAs around MCTs, compensating for low affinity and accelerating transport rates of BCKAs.

It currently remains unclear whether and how excretion of these metabolites could support glioblastoma growth and whether such effects require the exchange of BCKAs with cells of the tumor microenvironment, mainly tumor associated macrophages and microglia.

T17-07B

Identification of a gene mutated in 7.5% of anaplastic oligodendrogliomas

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Question: Anaplastic oligodendrogliomas (AO) are rare primary brain tumors, which are generally incurable, with heterogeneous prognosis and few treatment targets identified. Most

oligodendrogliomas have chromosome 1p/19q co-deletion, which is associated with a better prognosis. Recent high-throughput sequencing approaches have identified *IDH1* and *IDH2*, *CIC*, *FUBP1* and *TERT* (promoter) mutations. Identifying additional driver genes and altered pathways in oligodendroglioma offers the prospect of developing more effective therapies and biomarkers to predict individual patient outcome.

Methods: Here we performed whole-exome and transcriptome sequencing of anaplastic oligodendrogliomas to search for additional tumor driver mutations and pathways disrupted. We analyzed 51 AO by whole-exome sequencing and conducted targeted sequencing of a further 83 AO. Using all mutation results, we performed an analysis to identify pathways or gene ontologies that were significantly enriched in mutated genes. In parallel, we analyzed the histology and patient survival for respectively 73 and 69 of the tumors.

Results: We identified somatic mutations in *CIC* and *FUBP1*, as previously reported. Importantly however, we also identified recurrent mutations in a gene not previously associated with oligodendrogliomagenesis, which encodes a bHLH transcription factor involved in neural development. The overall frequency of AOs bearing mutations in this gene was of 7.5%. Interestingly, 80% of mutations identified occur in either the bHLH domain, important for function as a transcription factor, or are frame shift mutations leading to a truncated protein lacking this domain. We show that these mutations compromise the transcriptional activity and lead to protein accumutation. Furthermore, they are associated with a more aggressive tumor type.

Conclusions: Our data are compatible with an haplo-insufficient tumor suppressor function for this transcription factor. Intriguingly, haploinsufficiency for this gene was also reported in a developmental disorder. Together these data raise the possibility that carriers of germline mutations of this gene might be at an increased risk of developing AO. Our analysis provides further insights into the unique and shared pathways driving oligodendrogliomagenesis. To our knowledge this study represents the largest sequencing study of AO conducted to date.

T17-08B

EGFR expression confers stem cell-like properties to human SVZ progenitors and gliomas

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The Epidermal Growth Factor Receptor (EGFR) is strongly implicated in the migration and proliferation of neural/glial progenitors and is overexpressed in many low-grade and most high-grade gliomas, even in the absence of amplification or activating mutations of its gene. The relationship of EGFR to gliomagenesis is still poorly understood. We and others have shown that EGFR is highly expressed in human germinal matrix (GM) progenitors, and is largely silenced by adulthood, except for notable localization to a subset of cells residing in the adult subventricular zone (SVZ). Unlike in adult rodent SVZ, where EGFR labels actively dividing stem cell astrocytes and transit amplifying cells, we see that human EGFR-positive adult SVZ cells are non-proliferating under normal conditions. The phenotypic and functional properties of these cells have not been previously thoroughly analyzed. Here we undertook a detailed in vivo and in-vitro characterization of EGFR-positive cells in the developing and adult SVZ using fresh human postmortem brain tissue, hypothesizing that EGFR-positive cells harbor stem cell-like characteristics, which may implicate them in gliomagenesis.

For the in vivo characterization, we performed detailed confocal immunofluorescence analysis using a panel of markers for glial/neuronal lineage differentiation and proliferation. In order to characterize the stem cell behavior of these cells in vitro, we developed a unique, human-based protocol for acute isolation of EGFR expressing cells in fresh post-mortem human tissue, adapted from established methods in mice. We found that EGFR-positive but not EGFR-negative cells purified from fetal and infant SVZ show stem-cell characteristics in vitro, including self-renewal as primary/secondary neurosphere formation and multipotential for trilineage differentiation. We have previously reported on a strikingly similar, activating epigenetic landscape at *EGFR* in human GM progenitors and gliomas

that express the gene, implicating the GM as a putative glioma region of origin. Here we demonstrate the ability to also isolate EGFR-positive and negative glioma subpopulations, and further show that similarly to GM progenitors, only EGFR-expressing tumor cells are capable of self-renewal in vitro. Our findings suggest that EGFR expression confers stem cell-like characteristics to human progenitor and glioma cells, reinforces the notion that human neural stem cells can contribute to tumor formation, and hints that maintenance of EGFR expression may play a role in gliomagenesis.