Investigation of Tick Neurobiology toward New Control Measures of Ticks and Tick-borne Pathogens

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Investigation of Tick Neurobiology toward New Control Measures of Ticks and Tick-borne Pathogens

Dossier de candidature
à l'Habilitation à
Diriger des Recherches

Présenté par

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Team: MiTick
Group: NeuroPaTick

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1. RESUME

English:

Ticks and tick-borne pathogens represent a very serious, ever-increasing problem to both human and animal health. Ticks are second only to mosquitoes as vectors of human diseases and represent the most important arthropods affecting the cattle industry, as more than 80% of the world's cattle population is affected by tick-borne diseases. Current tick management methods are clearly inadequate and the key factors driving the emergence of tick-borne diseases are poorly defined. There is no doubt that effective tick and tick-borne disease control strategies are currently hampered due our limited understanding of their physiology.

Since the beginning of my professional career starting by PhD study, (2002, Slovakia) till my current principal investigator position in France I was fortunate to stay in the same research direction focusing on highly specific field named: ticks molecular neurophysiology. Currently, only few laboratories around the world are capable to study tick neurophysiology, while most of the invertebrate neurophysiologists focus on the model organisms like *Drosophila melanogaster* or *Caenorhabditis elegans*. This can be advocated by the complicated ectoparasitic life style of ticks resulting in significant difficulties to establish efficient rearing colonies of these medically important arthropods in the laboratory. On the other hand, tick community focuses studies primarily to tick morphology, taxonomy, behavior, epidemiology or interaction with their hosts or pathogens they transmit. Specifically the goal of their research has been developing treatments to block tick transmission of disease-causing pathogens. However, from the biological point of view, the researchers are beginning to recognize that ticks are organisms endowed with an incredibly unique physiology and behavior, including capabilities not found in any other blood feeding arthropods.

My current laboratory (www.neuropatick.com) in UMR-BIPAR (Maisons-Alfort, France) examining the tick neural signaling pathways is placing an attention to different aspect of tick physiology directly linked to the activities of their neural system. Using the highly innovative cutting edge tools we are fortunate to explore this fascinating and burgeoning field, which sheds a completely new light on unique aspects of tick biology. We anticipate that identifying specific components of the tick signaling systems will present key targets for developing novel tick management strategies, and which could be applicable to a broad spectrum of different tick-host pathogen interactions, rather than just unique solutions for each pathogen.

My scientific trajectory, including the current research direction, in this highly specific/unique research field are described below via the detailed CV and the most significant past and current projects.

***French*:**

Les tiques et les agents pathogènes transmis par les tiques représentent un problème très sérieux et de plus en plus important pour la santé humaine et animale. Les tiques sont les deuxièmes vecteurs de maladies humaines après les moustiques et représentent les arthropodes les plus importants affectant l'industrie de l'élevage bovin, car plus de 80% du cheptel mondial est touché par des maladies transmises par les tiques. Les méthodes actuelles de gestion des tiques sont clairement inadéquates et les principaux facteurs à l'origine de l'émergence de maladies transmises par les tiques sont mal définis. Il ne fait aucun doute que les stratégies efficaces de contrôle des tiques et des maladies transmises par les tiques sont actuellement entravées par notre compréhension limitée de leur physiologie.

Depuis le début de ma carrière professionnelle, commençant par un doctorat (2002, Slovaquie) jusqu'à mon poste actuel de coordinateur scientifique en France, j'ai eu la chance de rester dans la même thématique de recherche en me focalisant sur un domaine très spécifique : la neurophysiologie moléculaire des tiques. Actuellement, seuls quelques laboratoires dans le monde sont capables d'étudier la neurophysiologie des tiques, tandis que la plupart des neurophysiologistes spécialistes des invertébrés privilégient les organismes modèles comme *Drosophila melanogaster* ou *Caenorhabditis elegans*. Cela peut être justifié par le mode de vie ectoparasitaire complexe des tiques, qui rend très difficile la mise en place au laboratoire de colonies d'élevage qui soient efficaces, pour ces arthropodes importants pour la recherche médicale. D'autre part, la communauté scientifique travaillant sur les tiques dirige principalement ses études sur la morphologie, la taxonomie, le comportement, l'épidémiologie ou l'interaction avec leurs hôtes ou les agents pathogènes qu'elles transmettent. Plus précisément, le but de leur recherche a été de développer des traitements pour bloquer la transmission d'agents pathogènes par les tiques. Cependant, du point de vue biologique, les chercheurs commencent à reconnaître que les tiques sont des organismes dotés d'une physiologie et d'un comportement incroyablement uniques, y compris des capacités que l'on ne trouve chez aucun autre arthropode hématophage.

Mon laboratoire actuel (www.neuropatick.com) à l'UMR-BIPAR (Maisons-Alfort, France), qui étudie les voies de signalisation neuronale des tiques, porte une attention particulière à différents aspects de la physiologie des tiques directement liés aux activités de leur système neuronal. En utilisant les outils de pointe hautement innovants, nous avons la chance d'explorer ce domaine fascinant et en plein essor, qui apporte un éclairage complètement nouveau sur des aspects uniques de la biologie des tiques. Nous prévoyons que l'identification des composants spécifiques des systèmes de signalisation des tiques présentera des cibles clés pour le développement de nouvelles stratégies de gestion des tiques, et qui pourraient être applicables à un large éventail d'interactions tiques-hôtes pathogènes, plutôt que de simples solutions uniques pour chaque pathogène.

Mon parcours scientifique, y compris l'orientation actuelle de mes recherches, dans ce domaine de recherche très spécifique et unique est décrit ci-dessous dans le CV détaillé et les projets les plus importants dans lesquels j'ai été et je suis impliqué.

2. CURRICULUM VITAE

2.1 EDUCATIONAL BACKGROUND

PhD. (Doctor of Philosophy) in Zoology, Comenius University in Bratislava, Slovak Republic

- Date: 23.02.2007
- Thesis title: *Neuropeptides in the Nervous and Endocrine Organs of Ticks*
- Supervisors: Mirko Slovák, PhD., Institute of Zoology, Slovak Academy of Sciences, Bratislava, Slovak Republic) and Dušan Žitňan, DrSc., Institute of Zoology, Slovak Academy of Sciences, Bratislava, Slovak Republic
- Place of study: Institute of Zoology, Slovak Academy of Sciences, Bratislava, Slovak Republic

RNDr. (Doctor of Natural Sciences, from Latin: *Rerum Naturalium Doctor*) in Biology, Postgraduate academic degree, Pavol Jozef Safarik University, Košice, Slovak Republic

- Date: 11.06.2004
- Thesis title: *Interaction of Males and Females of Ticks During the Feeding on the Host*
- Supervisor: Mirko Slovák, PhD., Institute of Zoology, Slovak Academy of Sciences, Bratislava, Slovak Republic

Mgr. (Magister – equivalent of Master "MSc" degree), Pedagogy in General Education – Biology and Environmentalism, Constantine the Philosopher University, Faculty of Natural Sciences, Nitra, Slovak Republic

- Date: 18.06.2002
- Thesis title: *Analyses of the Landscape Structure of the Prievidza County (Slovakia)*
- Supervisor: Mgr. Jaroslav Košťál, PhD., Constantine the Philosopher University, Faculty of Natural Sciences, Nitra, Slovak Republic

2.2 PROFESSIONAL EXPERIENCES

Feb. 2016 – presence

- **Principal Investigator CRCN** (100% time research position, CDI), French National Research Institute For Agriculture, Food And Environment (INRAE), Dept. of Animal Health, Joint Research Unit of Molecular Biology and Immunology of Parasites (UMR-BIPAR, Anses, INRAE, EnvA) in Maisons-Alfort.
- **Team leader** MiTick (Microorganismes & Tiques) since 21. December 2020, grouping all tick researchers in UMR-BIPAR that includes 7 permanent researchers (INRAE, ANSES, National Veterinary School of Alfort-ENVA), 5 technicians, 3 engineers and current, postdoctoral researchers, PhD and Master 2 students (annually together about 20 persons per annum)
- **Group leader** NeuroPaTick (Tick neurophysiology & Tick-host-pathogen interactions www.neuropatick.com) since January 2020

Jun. 2014 – Jan. 2016

- **Postdoctoral Fellow**, UMR-BIPAR, Maisons-Alfort (France), Supported by Pasteur Institute, Paris, LabEx, Integrative Biology of Emerging Infectious Diseases (LabEx IBEID), Affiliated with ANSES - Animal Health Laboratories.
  Topic: *Identification of the molecules contributing to tick-pathogen transmission and development of new generation vaccines*

Supervisor: Sarah Bonnet, PhD (INRAE, UMR-BIPAR)

Oct. 2011 – Jun. 2014

- **Research Assistant Professor,** Kansas State University (USA), Arthropod Molecular Physiology Laboratory, Dept. of Entomology, Manhattan KS, USA, Supported by NIAID/NIH.
  Topic: *Signalling pathways for tick salivary secretion*
  Supervisor: Prof. Yoonseong Park, (Kansas State University)

Apr. 2007 – Oct. 2011

- **Postdoctoral Research Associate**, Kansas State University (USA), Arthropod Molecular Physiology Laboratory, Dept. of Entomology, Manhattan KS, USA Supported by NIAID/NIH and NCRR/NIH
  Topic: *Neuropeptidergic control of the salivary gland in the black- legged tick, Ixodes scapularis*
  Supervisor: Prof. Yoonseong Park, (Kansas State University)

Oct. 2006 – Mar. 2007

- **Postdoctoral Research Associate**, Slovak Academy of Sciences (Slovakia), Ticks-Insects Laboratory, Institute of Zoology, Department of Entomology, Bratislava, Slovak Republic
  Topic: *Neuropeptides in the nervous and endocrine organs of ticks*
  Supervisor: Mirko Slovák PhD., Dušan Žitňan, DrSc., (Slovak Academy of Sciences, Institute of Zoology)

2.3 TEACHING ACTIVITIES

INRAE CRCN (France)

- Invited guest lecturer: *The physiology of tick saliva secretion*. Biology and Health, Pathogen interactions, Circulation of pathogens and infectious diseases and parasitic animals" Master 2 students (M2-UVSQ – Paris Saclay, MSBio907), *Paris-Saclay Versailles Saint- Quentin University of Science and Technology* (France) at campus of National Veterinary School of Alfort (ENVA). (1 hour and 30min/year, 2016-2020)
- Invited guest lecturer National Veterinary School of Alfort (ENVA, Maisons-Alfort). *Hard ticks and their feeding biology*. 2nd year veterinary students. Course: Acarology and Parasitology (UC81). (1 hour in 2019)
- Invited guest lecturer: *Tick neurophysiology,* Module: Zoonoses liées aux tiques : impact pour l'Homme et l'Animal, une approche One Health., National Veterinary School of Alfort (ENVA, Maisons-Alfort), 27 November 2019. (2 hours)
- Invited guest lecturer: *Tick morphology and physiology*, (Graduate master and PhD student) Key Laboratory of Entomology and Pest Control Engineering, College of Plant Protection, Southwest University, Chongqing 400715, China, November 22. (1 hour in 2017)
- Invited guest lecturer: *Insight in to the immunohistochemistry and in situ hybridization laboratory techniques*. (Graduate master and PhD student) Key Laboratory of Entomology and Pest Control Engineering, College of Plant Protection, Southwest University, Chongqing 400715, China, November 22. (1 hour in 2017)

Research assistant professor and postdoctoral research associate (USA)

- Invited guest lecturer: *Insect Physiology,* Kansas State University (USA), (2hours, Spring 2014).
- Invited guest lecturer: *Biology of Disease Vectors of Human and Veterinary Importance,* Kansas State University (USA) (1 hour, Fall 2013)
- Invited guest lecturer: *Insect Physiology*, Kansas State University (USA), (2hours, Spring 2012)
- Invited guest lecturer: *Biology of Disease Vectors of Human and Veterinary Importance,* Kansas State University (USA) (1 hour, Fall 2011)
- Molecular Biology/Biochemistry - Laboratory Instructor 2009-2014. Supervision and instruction of graduate students and postdocs in molecular biology and biochemistry techniques (including immunohistochemistry, in situ hybridization, PCR, RNA, DNA isolation, confocal microscopy, bioinformatics, MALDI etc.), Kansas State University, (USA) (3-4 students/postdocs per semester)

PhD student (Slovakia)

- Histology & Organology – Class Teacher 2002-2003
  Comenius University, Faculty of Natural Sciences (Slovakia), (56 hours/two semesters total, 15 students per class)

2.4 OBTAINED FUNDING

Ladislav Šimo as Scientific Coordinator:

- **The National Veterinary School of Alfort (ENVA) (2017) 1.1.2017 – 31.1.2017**
  Role: Scientific Coordinator
  Amount: **8.529€**
  Title: "*Neurotransmitters signaling in tick salivary glands"*

- **National Institute of Agricultural Research (INRA) (2017) 1.1.2017 – 31.1.2017**
  Role: Scientific Coordinator
  Amount: **10.000€**
  Title: "*Cholinergic signaling in the salivary glands of Ixodes ricinus*"

- **Île-de-France DIM-1Health (NeuroPaTick) 28.10.2018- 27.10.2020**
  Role: Scientific Coordinator
  Amount: **103.600€** (to allocate the postdoctoral fellow)
  Axis2: Prevent and treat infectious diseases: https://www.dim1health.com/en/supported-projects/neuronal-basis-of-tick-pathogen-interactions/
  Title: Neuronal Basis for Tick-Pathogen Interactions (NeuroPaTick)

- **Laboratory of Excellence (LabEx), Integrative Biology of Emerging Infectious Diseases (IBEID) Pasteur Institute 2021-2024 granted for the MiTick team in UMR-BIPAR**
  Scientific coordinators: Sara Moutailler, Alejandro Cabezas-Cruz and Ladislav Šimo
  Role: Scientific coordinator of the 3rd aim (3 aims total)of the project (Impact of anti-tick microbiota and anti-gut GPCRs vaccines), also the member of the scientific team.
  Amount: **635.000€** (135.000€ ENVA /ANSES + 500.000€ LabEx IBEID Pasteur Institute)
  Title: New insights regarding tick co-infections?

Contribution to others funded projects:

- **Satt, Île-de-France (Visions) 2018 - 2020**
  Scientific coordinator: Sarah Bonnet (INRAE, UMR-BIPAR)
  Role: Member of the work package
  Amount: **275.400€**
  Title: Identification of tick molecules implicated in the pathogen transmission–next generation vaccine development
- **DIM-1Health (Région Ile-de-France) investments 2019**
  Scientific coordinator: Sarah Bonnet (INRAE, UMR-BIPAR)
  Role: Member of the work package
  Amount: **157.958 €**
  Title: Tiquarium » pour l'élevage d'espèces de tiques dures endémiques et invasives.
- **PARADIM investments (Région Ile-de-France) 2018**
  Scientific coordinator: Bruno Polack & Nadia Haddad (ENVA, UMR-BIPAR)
  Role: Member of the work package
  Amount: **98.720 €**
  Title: renforcement du potentiel –omique pour l'étude des agents pathogènes en santé animale a l'UMR BIPAR et sur le Site d'Alfort
- **DIM-1Health investments 2017**
  Scientific coordinator: Dr. Gregory Karadjian (ANSES, UMR-BIPAR)
  Role: Member of the work package
  Amount: **232.323€**
  Title: Etude des agents pathogènes en Santé Animale: installation d'un microscope confocal sur le site d'Alfort
- **DIM-1Health investments 2017**
  Scientific coordinator: Prof. Henri-Jean Boulouis (ENVA, UMR-BIPAR)
  Role: Member of the work package
  Amount: **238.113€**
  Title: Etude des agents pathogènes en Santé Animale et Humaine: plateforme de spectrométrie masse -MALDI-TOF sur le site d'Alfort

2.5 ORGANISATION OF INTERNATIONAL CONFERENCES

- Co-organization (with Dr. A. Cabezas-Cruz, INRAE) of the first French-Czech Tick Meeting (FCTM1) , at the ANSES campus site in Maisons-Alfort (24-26 October 2019). The conference was the first meeting (27 speakers) of tick researchers from Biological Centre of Czech Academy of Scientist, Ceske Budejovice and UMR-BIPAR, UMR-Virologie and unit of Epidemiology from Maisons-Alfort. The meeting strengthened the establishment of further collaboration in tick research in the future, in the context of a Memorandum of Understanding (M.O.U.) between the two countries. https://www.vet-alfort.fr/actualites-de-l-ecole/deux-jours-consacres-aux-tiques; https://www.neuropatick.com/fctm-1

2.6 EDITORIAL AND SCIENTIFIC ADVISORY BOARD ACTIVITIES

- An special issue guest editor of Pathogens journal (MDPI, Multidisciplinary Digital Publishing Institute), special issue "*New Frontiers in Tick Research*" January 2020-December 2021
  https://www.mdpi.com/journal/pathogens/special_issues/New_Frontiers_in_Tick_Research
- A member editorial board of Pathogens journal (MDPI), section "*Ticks*" from 2021. https://www.mdpi.com/journal/pathogens/sectioneditors/Ticks

- A member of editorial board of section *Invertebrate Physiology* in Frontiers in Physiology. Since 2021
- A member of editorial board of section *Insect Physiology* in Frontiers in Insect Science. Since 2021
- An special issue guest editor of Pathogens journal (MDPI, Multidisciplinary Digital Publishing Institute), special issue "*Tick Physiology and Behavior*" March 2021-March 2022 https://www.mdpi.com/journal/pathogens/special_issues/tick_physiology_behavior
- A member of International Advisory Board of Biology Centre of the Czech Academy of Sciences, Ceske Budejovice, Czech republic. April 2018 - June 2021 https://www.bc.cas.cz/cz/o-nas/international-advisory-board/. Main responsibilities include advisory and evaluation activities of the international performance of the Biology Centre and oversee the implementation of the IBERA project (Integration of the Biology Centre into the European Research Area) to meet the requirements of the human resources award program. The 10 members (high level specialists in different biology fields) IAB duties include evaluation of the scientific performance of the entire centrum (5 Research Institutes, total of 750 employees) and help of the centrum progress in the European Union research space for grant proposals.

2.7 PEER-REVIEW PUBLICATIONS

1. Boularias G., Azzag, N., Galon C., **Šimo L.**, Boulouis H-J., Moutailler S., 2021 High-throughput microfluidic real-time PCR for the detection of multiple microorganisms in Ixodid cattle ticks in northeast Algeria, *Pathogens 10*(3), 362, IF - 3.018

2. Mateos-Hernández L., Pipova N., Allain E., Henri C., Rouxel C., Lagree AC., Boulouis HJ., Valdes J., Alberti P., de la Fuente J., Cabezas-Cruz A.*****, **Šimo L.***, 2021 Enlisting the *Ixodes scapularis* Embryonic ISE6 Cell Line to Investigate the Neuronal Basis of Tick-Pathogen Interactions, *Pathogens 10* (1), 70 **(*corresponding author),** IF - 3.018

3. Mateos-Hernández L., Obregon D., Maye J., Borneres J., Versille N., de la Fuente J., Estrada-Peña A., Hodžić A., **Šimo L**., Cabezas-Cruz A., 2020. Anti-tick microbiota vaccine impacts Ixodes ricinus performance during feeding. *Vaccines*, *8*(4), 702, IF - 4.086

4. Almazan C.*, **Šimo, L**.*, Fourniol L., Rakotobe S., Bornéres J., Cote M., Peltier S., Maye J., Versille N., Richardson J., Bonnet S., 2020. Multiple antigenic peptide-based vaccines targeting Ixodes ricinus neuropeptides induce a specific antibody response but do not impact tick infestation. *Pathogens*, 9 (11), 900. (*equal contribution), IF - 3.018

5. Mateos-Hernandez L., Defaye B., Vancová M., Hajdusek O., Sima R., Park Y., Auttoui H., **Šimo L.***, 2020. Cholinergic axons regulate type I acini in salivary glands of Ixodes ricinus and Ixodes scapularis ticks. *Scientific Reports* 10, 16054. **(*corresponding author),** IF - 3.998

6. Almazan C., Fourniol L., Rakotobe S., **Šimo L.,** Bornéres J., Cote M., Peltier S., Maye J., Versille N., Richardson J., Bonnet S., 2020. Failed disruption of tick feeding, viability and molting after immunization of mice and sheep with recombinant Ixodes ricinus salivary proteins IrSPI and IrLip1. *Vaccines* 8, 475, IF - 4.086

7. Mateos-Hernández L., Rakotobe S., Defaye B., Cabezas-Cruz A., Šimo L.*, 2020. A capsule-based model for immature hard tick stages infestation on laboratory mice. *J. Vis. Exp.* (140), e57994 **(*corresponding author)**, IF – 1.35

8. Vancová M., Bílý T., **Šimo L.,** Touš J; Horodyský P., Ruzek D., Novobilský A., Salát J., Strnad M, Sonenshine D., Grubhoffer L., Nebesářová J., 2020. Three-dimensional reconstruction of the feeding apparatus of the tick Ixodes ricinus (Acari: Ixodidae). *Scientific Reports* 10:165, IF - 3.998

9. Park Y.*, Kim D., Boorgula G., Schutter K., Smagghe G., **Šimo L.**, Archer-Hartmann S., P. Azadi, 2020. Alpha-gal and cross-reactive carbohydrate determinants in the N-glycans of salivary glands in the lone star tick, Amblyomma americanum. *Vaccines*, (8, 18), IF - 4.086

10. Veenstra J. A. and **Šimo L.** 2020. The TRH-ortholog EFLamide in the migratory locust. *Insect Biochemistry and Molecular Biology*, 116:103281, IF - 3.827

11. Blisnick A., **Šimo L.**, Grillon C., Fasani F., Brûlé S., Le Bonniec B., Prina E., Marsot M., Relmy A., Blaise-Boisseau B., Richardson J., Bonnet S., 2019. The immunomodulatory effect of IrSPI, a tick salivary gland serine protease inhibitor involved in Ixodes ricinus tick feeding. *Vaccines*, 7, 148, IF - 4.086

12. Vancová M., Bílý T., Nebesářová J., Grubhoffer L., Bonnet S., Park Y., **Šimo L.***, 2019. Ultrastructural mapping of salivary gland innervation in the tick *Ixodes ricinus*. *Scientific Reports* 9:6860 **(*corresponding author),** IF - 3.998

13. Lejal E., Moutailler S., **Šimo L.**, Vayssier-Taussat M., Pollet T., 2019. Tick-borne pathogen detection in midgut and salivary glands of adult Ixodes ricinus. *Parasites & Vectors* 12(1):152, IF – 3.43

14. Cabezas-Cruz A.[1], Espinosa PJ.[1], Alberdi P.[1], **Šimo L.**[1], Valdés JJ., Mateos-Hernández L., Contreras M., Rayo MV., de la Fuente J., 2018 Tick galactosyltransferases are involved in α-Gal synthesis and play a role during Anaplasma phagocytophilum infection and Ixodes scapularis tick vector development. *Scientific Reports* 8(1): 14224, ([1]equal contribution), IF - 3.43

15. Almazán C., Bonnet S., Cote M., Slovák M., Park, Y., **Šimo* L.**, 2018. Versatile Model of Hard Tick Infestation on Laboratory Rabbits. *J. Vis. Exp*. (140), e57994, **(*corresponding author)**, IF – 1.35

16. Kim D.[1], **Šimo L.**[1], Vancova M., Urban J., Park Y., 2012 Neural and endocrine regulation of osmoregulatory organs in tick: Recent discoveries and implications. *Gen and Comp Endocrinol.* S0016-6480(18)30327-7, 2018 **([1]equal contribution)**, IF - 2.426

17. Kim D., **Šimo L.**, Park Y., 2018 Molecular characterization of neuropeptide elevenin and two elevenin receptors, IsElevR1 and IsElevR2, from the blacklegged tick, Ixodes scapularis. *Insect Biochem Mol Biol*. 101:66-75, 2018, IF - 3.827

18. **Šimo L.**, Kazimirova M., Richardson J., Bonnet S., 2017 The essential role of tick salivary glands and saliva in tick feeding and pathogen transmission; 7: 281, *Frontiers in Microbiology*, 2017, IF - 4.235

19. Gulia-Nuss M., et al., (Ixodes scapularis genome sequencing consortium), 2016 Genomic insights into the Ixodes scapularis tick vector of Lyme disease. *Nature Communications*. 7:10507, doi:10.1038/ncomms10507, IF - 12.121

20. Avila L. A. ,Aps L. R. M . M., Sukthankar P., Ploscariu N., Guldur S., **Šimo L.**, Szoszkiewicz R., Park Y., Lee S. Y., Iwamoto T., Tomich J. M., 2015 Branched amphiphilic cationic oligo-peptides form peptiplexes wih DNA: A study of their biophysical properties and transfection efficiency. *Molecular Pharmaceutics* 12 (3), pp 706–715, IF - 4.321

21. Roller L[1]., **Šimo L[1].**, Mizoguchi A., Slovak M., Park Y. Žitňan D., 2015 Orcokinin-like immunoreactivity in central neurons innervating the salivary glands and hindgut of Ixodid ticks.

Cell and Tissue Research 360(2):209-22, Including **"front cover page"** of the issue. **([1] equal contribution)**, IF - 3.043

22. Kim D., **Šimo L.**, Park Y., 2014 Orchestration of salivary secretion mediated by two different dopamine receptors in the blacklegged tick, Ixodes scapularis, *Journal of Experimental Biology* 217:3656-3663, Selected by editor for **"inside JEB"** section article, IF - 3.014

23. **Šimo L*.** and Park, Y*., 2014 Neuropeptidergic control of the hindgut in the black legged tick Ixodes scapularis. *International Journal for Parasitology* 44(11):819-826, Selected as an **"editors' choice article"** as well as including **"front cover page"** of the issue, **(* corresponding author)**, IF - 3.782

24. Koči J., **Šimo L.**, Park, Y., 2014 Autocrine/paracrine dopamine in the salivary glands of the blacklegged tick Ixodes scapularis. *Journal of Insect Physiology* 62:39-45, IF – 2.246

25. **Šimo L.**, Koči J., Kim D. H., Park Y., 2014 Invertebrate specific D1-like dopamine receptor in control of salivary glands in the black-legged tick Ixodes scapularis, *Journal of Comparative Neurology* 522(9):2038-52, IF - 3.331

26. Jiang H., Ikhagva A., Daubnerová I., Chae H., **Šimo L.**, Jung S. H., Yoonb Y. K., Lee N. R., Seong J. Y., Žitňan D., Park Y., and Kim Y. J., 2013 Natalisin, a new tachykinin-like signaling system, regulates sexual activity and fecundity in insects, *Proceedings of National Academy of Sciences* 110 (37), E3526-E3534, IF - 9.412

27. **Šimo L.**, Koči J., Park Y., 2013 The receptors for neuropeptides myoinhibitory peptide and SIFamide in the salivary glands of the blacklegged tick, Ixodes scapularis*, Insect Biochemistry and Molecular Biology* 43:376-387, IF - 3.827

28. Koči J., **Šimo L.**, Park Y., 2013 Validation of internal reference genes for real-time quantitative PCR studies in the tick Ixodes scapularis*, Journal of Medical Entomology* 50:79-84, IF - 1.712

29. **Šimo L*.**, Žitňan D. and Park Y*., 2011 Neural control of salivary glands in Ixodid ticks *Journal of Insect Physiology* 58:459–466, **(* corresponding author)**, IF - 2.246

30. **Šimo L.**, Koči J., Žitňan D. and Park Y., 2011 Evidence for D1 dopamine receptor activation by a paracrine signal of dopamine in tick salivary glands, *PLoS ONE* 6(1): e16158, IF - 2.740

31. Roller L., Žitňanová I., Dai L., **Šimo L.**, Park Y., Satake H., Tanaka Y., Adams M. E., Žitňan D., 2009, Ecdysis triggering hormone signaling in arthropods*, Peptides* 31(3):429-441, IF - 2,618

32. **Šimo L.**, Žitňan D. and Park Y., 2009 Two novel neuropeptides innervate the salivary glands in the blacklegged tick Ixodes scapularis*:* Myoinhibitory peptide and SIFamide, *Journal of Comparative Neurology* 517:551–563, Including **"front cover page"** of the issue, IF - 3.331

33. **Šimo L.,** Slovák M., Park Y. and Žitňan D., 2009 Identification of a complex peptidergic neuroendocrine network in the hard tick, Rhipicephalus appendiculatus*, Cell and Tissue Research* 335(3):639-655, IF - 3.043

34. Peterková K., Vančova I., Hajnická V., Slovák M., **Šimo L.** and Nuttall P. A., 2008 Immunomodulatory arsenal of nymphal ticks, *Medical and Veterinary Entomology,* 22, 167-171, IF -2.178

35. Vančová I., Slovák M., Hajnická V., Labuda M., **Šimo L.**, Peterkova K., Hails R. S., Nuttall P. A., 2007 Differential anti-chemokine activity of Amblyomma variegatum adult ticks during blood-feeding, *Parasite Immunology* 29 (4),169–177, IF - 2.054

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2.8 BOOK CHAPTERS

- **Šimo L.,** 2021 Climatic influence on tick neurobiology. In: *Climate, Ticks and Disease*, Ed. Patricia Nuttall, CAB international. (In press)

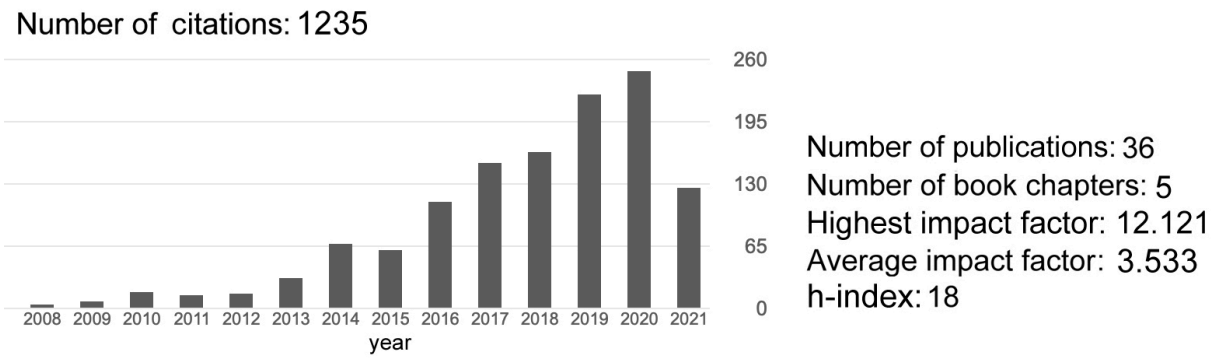
- Sonenshine E. D. and **Šimo L.**, 2021 Biology and Molecular Biology of Ixodes scapularis In: *Lyme Disease and Relapsing Fever Spirochetes: Genomics, Molecular Biology, Host Interactions and Disease Pathogenesis.* Eds.: Justin D. Radolf and D. Scott Samuels, Caister Academic, UK, pp.339-368

- Bonnet S., Kazimirova M., Richardson J., **Šimo L.**, , 2018 Tick saliva and its role in pathogen transmission. In: *Skin and arthropod vectors*, Ed.: N. Boulanger 121:191, Elsevier, UK, pp121-191

- **Šimo L*.,** Sonenshine D., Park Y. and Žitňan D., 2014 The Nervous and Sensory Systems: Structure, Function, Proteomics and Genomics, *Biology of Ticks Volume 1*. EDs: D. E. Sonenshine and R. M. Roe. Oxford University Press, Inc., 198 Madison Avenue, New York, pp.309-367

- Zďárek J., Takáč P., Keszeliová D., **Šimo L.**, Roller L., Cvačka J., Šanda M., 2012 Parturition in tsetse flies: Endocrine control, Quality Control for Expanded Tsetse Production, Sterilization and Field Application, In: *Insect Pest Control Section, International Atomic Energy Agency*, Vienna, Austria, pp.63-97

2.9 PUBLICATION OUTCOME SUMMARY



2.10 SUPERVISORY ACTIVITIES

- **2021-2022 - PhD Student**, 50% co-supervision, Alejandra Wu Chuang, University Paris-Saclay, Director of the thesis: Dr. Alejandro Cabezas-Cruz, INRAE),
  Project: "*Tick anti-microbiota vaccine"*
- **2021 – Master 1 Student**, Fetta Guerrib, University Paris-Saclay
  Project: "*Functional characterization of neuropeptides receptors in tick salivary glands"*
- **2021 – 2023 - Post-Doctoral fellow**, Dr. Lourdes Mateos-Hernandez, ANSES, Funding: (LabEx) Integrative Biology of Emerging Infectious Diseases (IBEID), Pasteur Institute Paris.
  Project : "*New insights regarding tick co-infections*?*"*
- **2021 – Master 2 student**, (co-supervision with Dr. Gregory Karadjian, UMR BIPAR) Caina Ning, Paris-Saclay, Biologie-Santégents Infectieux : interaction avec leur hôtes et l'environnement
  Project: "*Etude des Neuropeptides de Trichinella spiralis (NeuropepTrich)*"
- **2017** - **Master 2 student**, Baptiste Defaye, University of Limoges, Life's Sciences and Health,
  Project: "*Neuropeptidergic and cholinergic system in Ixodid ticks*"
  Number of publications: **2**
- **2018 - 2020 - Post-Doctoral fellow**, Dr. Lourdes Mateos-Hernandez INRAE, Funding: (DIM-One Health, NeuroPaTick)
  Project: *"Neural Basis of Tick Pathogen Interactions"*
  Number of publications: **3**
- **Collaborative project**, Dr. Maria Vancova, Biology Centre, Czech Academy of Sciences, Czech Republic.
  Project: "*Ultrastructural mapping of innervation of the tick salivary glands*"
  Number of publications: **1**
- **2018 - Post-Doctoral fellow**, Dr. Consuelo Almazan, ANSES, UMR-BIPAR
  Project: "*Rabbit model for the tick feeding*"
  Number of publications: **1**
- **2017 - Visiting Post-Doctoral fellow**, Dr. Natalia Pipová, Pavol Josef Safarik University, Slovakia
  Project: *"Neuropeptidergic system in the tick Ixodes ricinus"*
  Number of publications: **1**

2.11 SUPERVISED RESEARCH-PUBLICATIONS (Supervised persons are in grey background)

- Mateos-Hernández L., Pipova N., Allain E., Henri C., Rouxel C., Lagree AC., Boulouis HJ., Valdes J., Alberti P., de la Fuente J., Cabezas-Cruz A.*****, **Šimo L. *** 2020 Investigating the Neuronal Basis of Tick-Pathogen interactions using the *Ixodes scapularis* Embryonic ISE6 Cell Line, *Pathogens 10* (1), 70, doi.org/10.3390/pathogens10010070, **(*corresponding author)**

- Mateos-Hernandez L., Defaye B., Vancová M., Hajdusek O., Sima R., Park Y., Auttoui H., **Šimo L.*** 2020. Cholinergic axons regulate type I acini in salivary glands of Ixodes ricinus and Ixodes scapularis ticks. *Scientific Reports* 10, 16054, **(*corresponding author)**

- Mateos-Hernández L., Rakotobe S., Defaye B., Cabezas-Cruz A., **Šimo L.*** 2020. A capsule-based model for immature hard tick stages infestation on laboratory mice. *J. Vis. Exp.* e61430, **(*corresponding author)**

- Vancová M., Bílý T., Nebesářová J., Grubhoffer L., Bonnet S., Park Y., **Šimo L.*** 2019. Ultrastructural mapping of salivary gland innervation in the tick *Ixodes ricinus*. *Scientific Reports* 9:6860, **(*corresponding author)**

- Almazán C., Bonnet S., Cote M., Slovák M., Park Y., **Šimo L.*** 2018 A Versatile Model of Hard Tick Infestation on Laboratory Rabbits. *J. Vis. Exp.* (140), e57994, **(*corresponding author)**

2.12 INVITED PRESENTATIONS

- **Šimo, L.** (2019) Unlocking the mechanisms of tick salivary gland control, promoting the development of tick and tick borne disease control measures. 13th International Symposium of Ticks and Tick Borne Diseases (13th STTBD), Weimar, Germany, 28-30 March (**keynote speaker**)
- **Šimo, L. (**2017) Identifying vulnerable spots in tick salivary gland physiology, International Symposium for New Technology in Arthropods Pest Management (ISNTAPM), Southwest University, Chongqing, China, 16-22 November
- **Šimo, L.** (2016) Neuropeptidergic control of the hindgut of the black-legged tick Ixodes scapularis, International Conference of Cell Biology. Prague, Czech republic
- **Šimo, L.** (2015) Neural control of tick salivary glands in Ixodid ticks, UMR-CMAEE research unit, Campus International de Baillarguet, Montpellier, France
- **Šimo, L.** (2014) Neural control of tick salivary glands: "The secret to tick spit". Vector biology seminar. Pasteur Institute Paris, France
- **Šimo, L**. (2013) Neural control of tick salivary glands: "The secret to tick spit". Invited seminar, Department of Biology, York University, Toronto, Canada
- **Šimo, L.,** (2011) Koči, J., Park, Y. Control of salivary glands in black-legged tick Ixodes scapularis. Society of Experimental Biology annual meeting, Glasgow, Scotland, UK

2.12 CONFERENCES

Oral presentations:

- Lemasson M., Caignard G., Unterfinger Y., Attoui H., Bell-Sakyi L., Moutailler S., **Šimo L.**, Johnson N., Vitour D., Saleh D.C., Richardson J., Lacour S.A., Dialogue moléculaire entre les virus de l'encéphalite à tique et louping ill avec leur vecteur tique Ixodes ricinus. Réunion annuelle Tiques et Maladies à Tiques, 22-23 Mars 2021. Visioconférence.
- Rispe C, Hervet C, Aury J-M, Labadie K, Thany S, **Šimo L.**, Charvet C, Neveu C and Plantard O, Deciphering the synganglion transcriptome of *Ixodes ricinus*, reunion annuelle groupe Tiques Et Maladies A Tiques, 22 et 23 Mai 2019, IMMUNINV, Montpellier, France
- Mateos-Hernandez L., Cabezas-Cruz A., **Šimo L.,** présentation orale : Neuronal Basis of Tick-Pathogen Interactions, reunion annuelle groupe Tiques Et Maladies A Tiques, 22 - 23 Mai 2019, IMMUNINV, Montpellier, France
- **Šimo L.,** Imaging approaches to study ticks signaling systems in UMR-BIPAR, Animation du department SA: Imagerie FIAP Jean Monnet, 27, April, 2018, Paris, France
- **Šimo, L.** Cholinergic Signaling In Salivary Glands Of The Tick *Ixodes ricinus*, Journées d'animation du Département Santé Animale, 8 au 11 octobre 2018, Nantes, France
- Pollet T., Lejal E., Marsot M., Moutailler S., Cosson J. F., Chalvet-Monfray K., **Šimo L.**, Vayssier-Taussat M., Un premier aperçu de la dynamique du pathobiome de la tique *Ixodes ricinus*, Journées d'animation du Département Santé Animale, 8 - 11 octobre 2018, Nantes, France
- Vancová M, **Šimo L**., Bílý T, Park Y, Nebesářová J Association of axonal processes with contractile cells in salivary glands of the tick *Ixodes ricinus* 19th International Microscopy Congress (IMC19), 9-14 September 2018, Sydney, Australia

- Blisnick A., **Šimo L.**, Caignard G., Le Bonniec B., Lacour S., Le Naour E., Cote M., Richardson J., Bonnet S. Characterization of IrSPI, an *Ixodes ricinus* serine protease inhibitor involved in tick feeding and in bacterial infection of salivary glands. 111th annual meeting of the German zoological society. Adaptations to Hematophagy in Blood-feeding Parasites symposium, 10-11st September 2018, Greifswald, Germany
- Blisnick A., **Šimo L.**; Caignard G.; Le Bonniec B., Lacour S., Le Naour E., Cote M., Richardson J., Bonnet S., Characterization of IrSPI, an *Ixodes ricinus* serine protease inhibitor involved in tick feeding and in bacterial infection of salivary glands. In ENVA doctoral days 2018, Maisons-Alfort, France
- Blisnick, A., **Šimo L.**; Caignard G.; Le Bonniec B., Lacour S., Le Naour E., Cote M., Richardson J., Bonnet S, 2018. Characterization of IrSPI, a tick serine protease inhibitor involved in tick feeding and in bacterial infection of salivary glands of *Ixodes ricinus*. In: Conference Young Researchers of Life Science, ENS, Paris, France.
- **Šimo L.,** Vancová M., Cholinergic signalling pathways in the salivary gland of *Ixodes ricinus*, 9th Tick and Tick-borne Pathogen Conference & 1st Asia Pacific Rickettsia Conference, September 2017 Cairns, Australia
- **Šimo L.,** Self-introduction: starting the tick physiology research in France, accomplishments and future plans, Tiques et Maladies-à-Tique, 2016, Sete, France
- Blisnick A., **Šimo L.,** Verheyden M., Bonnet S Characterization of IrSPI, a serine protease inhibitor implicated in tick feeding and bacterial infection of their salivary glands: preliminary results. Tiques et Maladies-à-Tique, *March 2016,* Sete, France
- **Šimo L.,** Neural Control of Tick Salivary Glands. Journées d'animation scientifique du département Santé Animale. May 2016, Chasseneuil-du-Poitou, France
- **Šimo L.** and Bonnet S., Identification of tick molecules that contribute to tick-borne pathogens transmission and development of next generation vaccines against their transmission. 4th Annual meeting of Integrative Biology of Emerging Infectious Diseases. Pasteur Institute, Laboratory of excellence. 2014, Paris, France
- **Šimo L.,** Kim D., Park Y., Neuropeptides, catecholamines and their receptors in the control of tick salivary glands, 8th International conference on ticks and tick-borne pathogens and 12th Biennial Conference of the Society for Tropical Veterinary Medicine, 2014, Cape Town, South Africa
- Kim D., **Šimo L.,** Park Y. Different physiological roles of two dopamine receptors in isolated salivary glands of the blacklegged tick, *Ixodes scapularis*. In Scientific Program for 24th ICE 2012 - New Era in Entomology, 2012, Daegu, South Korea
- Roller L., **Šimo L.**, Mizoguchi, A., Tanaka, Y., Slovák, M., Park, Y., Žitňan, D. Orcokinins are conserved peptides in innervation of the salivary gland and rectal sac of Ixodid ticks. In Scientific Program for 24th ICE, 2012 - New Era in Entomology, Daegu, South Korea
- Park Y. and **Šimo L.**, Neuropeptidergic control of the salivary gland in blacklegged tick *Ixodes scapularis*. Keynote speaker, International Symposium on Insect Physiology, Biochemistry, and Molecular Biology, 2011, Shanghai, China
- Park Y., and **Šimo L.**, The secret how ticks spit. Invertebrate Neuropeptide Conference, 2010, Merida, Mexico
- Žitňan D., **Šimo L.**, Roller L., Havlikova S., Slovák M., Park Y., Identification and function of neuroendocrine networks in ticks. XIII International Congress of Acarology, 2010, Recife, Brazil

- **Šimo L.,** and Park Y., Two novel neuropeptides in innervation of tick salivary glands: myoinhibitory peptide and SIFamide. The 6th Asia-Pacific Congress of Entomology, 2009, Beijing, China
- **Šimo L.,** Park Y., Žitňan D., Peptidergic innervation of salivary glands of the black-legged tick *Ixodes scapularis* (Say 1821). 6th International Conference on Arthropods: Chemical, Physiological, Biotechnological and Environmental Aspects. Stefan Kopeć Memorial Conference, 2009, Ochotnica dolna, Poland
- Roller L., Tanaka Y., Valachová-Spálovská I., **Šimo L.,** Žitňan, D., The analysis of neuropeptides encoded in the silkworm (*Bombyx mori*) genome. The Slovak and Czech National Conference "Biologically Active Peptides X", 2007, Prague, Czech Republic
- **Šimo L.,** Žitňan D., Takáč P., Slovák M., Vybrané hormóny hmyzu v centrálnej nervovej sústave a slinných žľazách kliešťa *Rhipicephalus appendiculatus* (Acarina, Ixodidae), 81. Fyziologické dni, 2005, Košice, Slovak Republic
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- **Šimo L.,** Slovák M., Žitňan D., Hajnická V., Prítomnosť hmyzých FMRF-amidov a tachykinínov u kliešťa *Rhipicephalus appendiculatus*. Zborník abstraktov, Česko-slovenská studentská vědecká konference, 2004, Brno Slovak Republic
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- Sláviková, M., Kocáková, P., Slovák, M., **Šimo L.**, Vančová, I., Hajnická, V., Inhibícia aktivity myších NK buniek slinami kliešťov rôznych druhov. 23rd Kongres Československé společnosti mikrobiologické, 2004, Brno, Slovak Republic
- **Šimo, L.,** Kocáková, P., Sláviková, M., Kubeš, M., Hajnická, V., Vančová, I., Slovák, M.: Vplyv niektorých biotických faktorov na sanie samičiek kliešťa *Dermacentor reticulatus* (Acarina: Ixodidae) v laboratórnych podmienkach. 9th Zoologická konferencia Feriancove dni, 2003, Bratislava, Slovak Republic

Poster displays:

- Rispe C, Hervet C, Aury J-M, Labadie K, Thany S, **Šimo L.**, Charvet C, Neveu C and Plantard O, What's in a tick "brain"? Transcriptome of *Ixodes ricinus* synganglions, Eight International Symposium on Molecular Insect Science, 7-10 July 2019 | Sitges, Barcelona, Spain
- Blisnick, A., **Šimo L.**, Caignard, G., Le Bonniec, B., Verheyden, M., Le Naour, E., Cote, M., Richardson, J., Bonnet, S., 2018. Survey of IrSPI, a tick serine protease inhibitor involved in tick feeding and in bacterial infection of salivary glands. In: The Batsheva de Rothschild Seminar on Frontiers in Parasitology, Tel-Aviv, Israel.
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- Blisnick A., **Šimo L.**, Verheyden, M., Cote, M., Naour, E.L., Bonnet, S., Characterization of IrSPI, a serine protease inhibitor implicated in tick feeding and bacterial infection of their salivary glands: preliminary results. Journée de la recherche à l'EnvA, June 2017, Maisonsl-Alfort, France
- **Šimo L.,** Vancová M., Cholinergic signaling pathways in the salivary glands of the tick *Ixodes ricinus*, Journée de la recherche à l'EnvA, June 2017, Maisonsl-Alfort, France
- Vancová M., **Šimo L.**, Bílý T., Nebesářová J. Spatial organization of peptide rgic axon terminals and myoepithelial cells in salivary glands of tick *Ixodes ricinus* 13th Multinational Congress on Microscopy, September 2017, Rovinj, Croatia
- Vancová M., **Šimo L.**, Bílý T., Nebesářová J., Three dimensional architecture of peptidergic axon terminals in salivary glands of tick *Ixodes ricinus* Conference ČSMS, Mikroskopie , May 2017, Bratislava, Slovakia
- Blisnick A., **Šimo L.**, Verheyden, M., Cote, M., Naour, E.L., Bonnet, S., Characterization of IrSPI, a serine protease inhibitor implicated in tick feeding and bacterial infection of their salivary glands: preliminary results, Journées d'animation scientifique du département Santé Animale**.** May 2016, Chasseneuil-du-Poitou, France
- Blisnick A., **Šimo L.**, Verheyden M., Cote M., Naour E.L., Bonnet S., Characterization of IrSPI, a serine protease inhibitor implicated in tick feeding and bacterial infection of their salivary glands: preliminary results, 6e journées d'étude Santé & Société d'Université Paris-Est « Agents infectieux et vaccins chez l'homme et l'animal » à l'EnvA, March 2016, Maisons-Alfort, France
- **Šimo L.,** Koči J., Park Y., Two novel neuropeptide receptors (Myoinhibitory peptide and SIFamide receptors) in neuronal controls of tick salivary glands 4rd Annual Arthropod Genomics Symposium Arthropod Genomics: New Approaches and Outcomes, 2011, Kansas City, Kansas, USA
- Koči J., **Šimo L.**, Park, Y. Biosynthesis of catecholamines in the salivary glands of tick: Tyrosine hydroxylase and Dopa decarboxylase, Entomological Society of America Annual Meeting, 2010, San Diego, California, USA
- **Šimo L.,** Koči J., Park Y., Dopamine receptor D1 in control of the salivary glands in the blacklegged tick *Ixodes scapularis*. Entomological Society of America, Annual Meeting, 2010 San Diego, California, USA

- **Šimo L.,** Koči J., Park Y., Dopamine receptor D1 in the salivary secretion of the blacklegged tick *Ixodes scapularis* 4th Annual Arthropod Genomics Symposium Arthropod Genomics: New Approaches and Outcomes, 2010, Kansas City, Kansas, USA
- **Šimo L.,** Park Y., Colocalization of two novel neuropeptides in innervation of salivary gland of the black legged tick *Ixodes scapularis* 3rd Annual Arthropod Genomics Symposium Frontiers in Arthropod Genomics, June 2009, Kansas City, Kansas, USA
- Žďárek J., Cvacka J., Sanda M., Keszeliová D., **Šimo L.** Roller L., and Takáč P., Parturition in the tsetse flies: endocrine control, Improved and Harmonized Quality Control for Expanded Tsetse Production, Sterilization and Field Application", 2008, Addis Ababa, Ethiopia
- **Šimo L.,** Park Y., Myoinhibitory peptide and pigment dispersing factor peptidergic inervation of the salivary glands of the blacklegged tick (*Ixodes scapularis* Say), Entomological Society of America, Annual Meeting, 2008, Reno, Nevada, USA *(poster)*
- **Šimo L.,** Park Y., Genomics and proteomics of neuropeptides in the black-legged tick *Ixodes scapularis*. Arthropod genomics symposium, 2008, Kansas City, Kansas, USA
- Vančová I., Slovák M., **Šimo L.**, Peterková K., Hajnická V., Anti-chemokine activities of *Amblyomma variegatum* female tick SGE in initial and final phases of feeding. 5th International conference on ticks and tickborne pathogens, 2005, Neuchatel, Switzerland
- **Šimo L.,** Žitňan D., Takáč P., Slovák M., Neuropeptides in hard ticks: immunocytochemistry and isolation. 5th International conference on ticks and tick-borne pathogens, 2005, Neuchatel, Switzerland
- **Šimo L.,** Žitňan D., Takáč P., Slovák M., Presence of insect myoinhibitory-, FMRFamide-and tachykinin-like substances in the CNS and salivary glands of the ixodid ticks. Biologically Active Peptides IX., 2005, Prague, Czech Republic
- **Šimo L.,** Žitňan D., Takáč P., Slovák M., Neuropeptides in hard ticks: immunocytochemistry and isolation. Kongres slovenských zoológov a konferencia Feriancove dni, 2005, Smolenice, Slovak Republic
- **Šimo L.,** Slovák M. a Žitňan D. Immunocytochemical mapping of insect FMRFamide- and Tachykininlike peptides in the ixodid tick *Rhipicephalus appendiculatus*. 3rd international and 28th European Peptide Symposium, 2004, Prague, Czech Republic

3. Research Activities

3.1 GRADUATE RESEARCH SLOVAK ACADEMY OF SCIENCES (PHD., SLOVAKIA)

3.1.1 Anti-chemokine effect of tick salivary gland extracts

The first project, I was involved in, was exploration the potential of tick salivary glands (SG) to subvert the host's cytokine activities. Dr. Patricia Nuttall from Oxford University (UK) served as the scientific advisor of this project. Ticks are well known for their immunomodulatory arsenal in their SG, and when fed on a host, the salivary secretion manipulates the host's immune responses (Šimo et al., 20017). Using ELISA techniques, we examined the ability of tick SG extracts to bind various chemokines in female, male, and nymphal ticks during blood feeding. These results revealed that ticks possess a range of anti-cytokine activities for controlling their host's immune response, which may facilitate pathogen transmission, and support the concept that ticks benefit from feeding together by exploiting molecular individuality. The work was published in *Parasite Immunology* (Vancová et al., 2007) and *Medical and Veterinary Entomology* (Peterková et al., 2008).

3.1.2 Neuropeptides in nervous and endocrine organs of ticks

The research that served as the basis for my PhD dissertation was focused on immunohistochemical mapping of the cells expressing different neuropeptides in the central nervous system and endocrine organs of ticks. In invertebrates neuropeptides and protein hormones (collectively, neuropeptides) along with their G-protein coupled receptors (GPCRs) are among the most important regulators directly or indirectly involved in nearly all of the diverse biological processes (Meeusen et al., 2003; Nässel, 2009). Neuropeptidergic cells synthesize the neuropeptide precursor, called prepropeptide that is usually composed of multiple copies of the same (or similar) neuropeptides. The prepropeptide signal peptide drives transportation for cleavage of the prepropeptide into smaller, mature neuropeptides. Then, these mature neuropeptides are packed into secretory vesicles that are eventually transported to axon terminals for exocytosis (Yeoh et al., 2017). The number of neuropeptide-encoding genes among invertebrates is more or less the same; however, their specific biological functions may differ among particular taxa (Schoofs et al., 2017).

Although the neuropeptides are crucial regulators of development and various physiological functions in invertebrates, before my research little was known about their identity, expression, or function in ticks. In my pioneer study I used 15 different antibodies against insect and crustacean neuropeptide molecules to visualize them in tick central nervous system, the synganglion (Fig. 1A-C), that has been proposed as the main source of tick neuropeptides. This organs has complex internal morphological organization (Fig. 1 B-D) and is composed of several sub-ganglions. The attention has been also paid on the SG (Fig. E-G) that may be a potential target of some neuropeptide signaling. In female ticks, grape-like SG clusters (Fig. 1 E-G) are composed of three types of spherical acini (I, II, and III hereafter) (Binnington, 1978). Each type of acini is composed of different type of cells reflecting their specific physiological roles (Fawcett et al., 1981). Agranular type I acini, located along the anterior portion of the main duct, are believed to be involved in osmoregulation when the tick is off the host (Kim et al., 2017; Needham et al., 1990), while granular types II and III produce copious saliva containing plethora of biologically active molecules manipulating host immune system (Fawcett et al., 1981; Šimo et al., 2017).

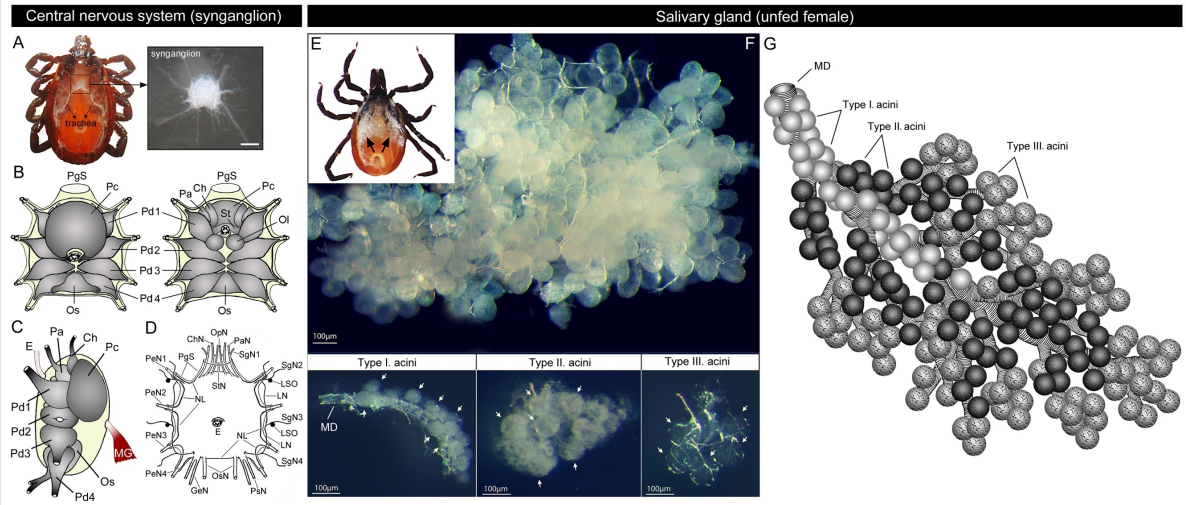


Figure 1. Left panel: Anatomy of the synganglion and associated peripheral nerves and organs. (**A**) Left, *R. appendiculatus* female tick showing the position of the synganglion. Scale bar is 200 µm. (**B**) Representations of various lobes in the dorsal (left) and ventral (right) view of the synganglion. Ch-cheliceral lobe, Ol-olfactory lobe, Os-opisthosomal lobe, Pa-palpal lobe, Pd1–4-pedal lobes 1–4, Pc-prothocerebral lobe, St-stomodeal lobe, PgS-periganglionic sheath. (**C**) Lateral view of the synganglion depicting positions of associated structures and organs; E esophagus, MD-midgut. (**D**) Representation of all peripheral nerves and lateral segmental organs (LSO). ChN-cheliceral nerve, GeN-genital nerve, LN-lateral nerve, OsN-opistosomal nerve, Op-N optical nerve, PaN-palpal nerve, PsN-paraspiracular nerve, PeN1–4-pedal nerves 1–4, StN-stomodeal nerve, SgN1–4-salivary gland nerves 1–4, E oesophagus. **Right panel:** Salivary gland of the *Ixodes ricinus* female. (**E**) *I. ricinus* female showing the position of salivary gland. (**F**) *I. ricinus* female salivary gland (upper panel), and separated acini types I, II and III (lower panels). (**G**) Schematic illustration of unfed *Ixodes* female salivary gland highlighting the position of acini types. MD main salivary duct. A and D are from Šimo et al., (2009a), F and G is from Sonenshine and Šimo, (2021).

The initial investigation was focused on the wholemount immunohistochemistry of the synganglion of the African tick species *Rhipicephalus appendiculatus* (Fig. 1.A) (Šimo et al., 2009a). The outcome of this approach uncovered the presence of a variety of entirely novel peptidergic neurons within different lobes of the synganglion (Fig. 2.) including multiple peptidergic axons exiting this organ. Among those, peptidergic innervation of *Rhipicephalus* SG was a completely unique discovery. I revealed that two neuropeptides myoinhibitory peptide (MIP) and pigment dispersing factor (PDF) are implicated in *Rhipicephalus* SG innervation (Šimo et al., 2009a) and further investigation confirmed these peptidergic innervations in several hard tick species (Šimo et al., 2012) suggesting a common control mechanism of this tissue in hard tick lineage (Fig. 3).

Specifically, antibody against MIP recognized a pair of giant protocerebral neurons (PcSG) as a source of the innervation of all (several hundreds) type II and III SG acini (Fig. 3A,B, D-F, J). The MIP-positive axon of each of these two neurons exit the synganglion via salivary nerve I (a branch of palpal nerve) and run along the main and adjacent SG ducts to reach both type II and III acini (Fig. 3A,J). Within each acinus the MIP-positive axons show typical peptidergic axon terminals shape (Fig. 3J). Interestingly morphological differences in the shape of these axon terminals were observed between species of the genus *Ixodes* and other hard tick genera (Fig. 3 J). On the other hand the antibody against PDF uncovered the presence of two pairs of opistosomal neurons sending tier axons to the salivary nerves II-IV (branches of lateral nerve (Fig. 3 C) and innervate exclusively acini type II (Fig. 3B, G-I, K). These novel data served as the basis for a peer-reviewed article, which was published in *Cell and Tissue Research* as the first report describing the complex neuropeptidergic network in ticks (Šimo et al., 2009a) and later review publication in *Journal of Insect Physiology* (Šimo et

al., 2012). Until this discovery, there was no evidence pertaining to neuropeptidergic control of tick SG, and catecholamines such as dopamine and norepinephrine remained as the sole candidates implicated in tick SG control (Kaufman, 1978, 1977; Kaufman and Harris, 1983; Sauer et al., 1995). Thus my study has opened a new area of neural regulation of tick SG.

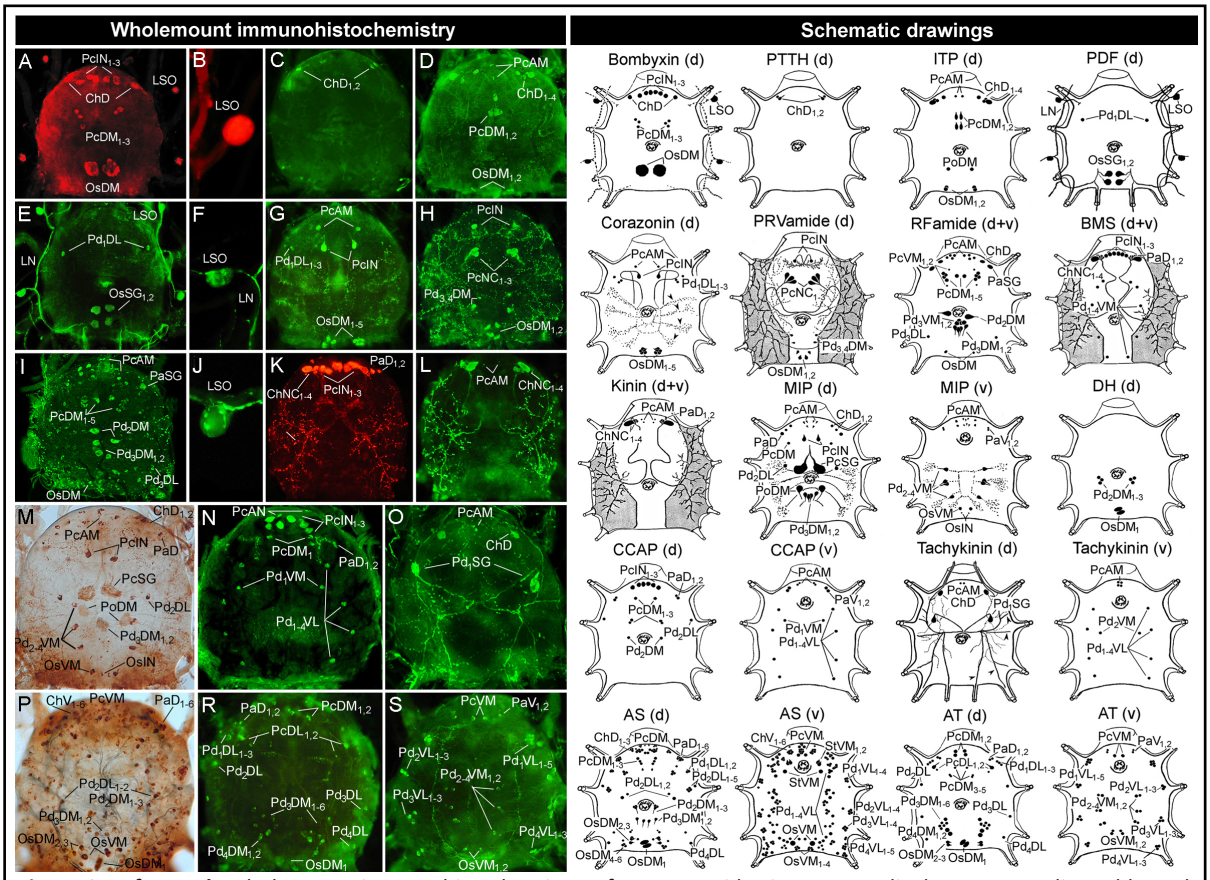


Figure 2. Left panel: Wholemount immunohistochemistry of neuropeptides in *R. appendiculatus* synganglia and lateral segmental organs. (**A, B**) Bombyxin-like immunoreaction (IR) in the synganglion and intrinsic cells of the lateral segmental organs (LSO). (**C**) Prothoracicotropic hormone (PTTH)-like IR in the anterior synganglion. (**D**) Ion transport peptide (ITP)-like IR in the synganglion. (**E, F**) PDF-like IR in the synganglion, lateral nerves (LN) and intrinsic cells of the LSO. (**G**) Corazonin-like IR in the synganglion. (**H**) PRVamide-like IR in the synganglion. (**I, J**) RFamide-like IR in the synganglion and putative neurohaemal axon terminals containing varicosities in the LSO. (**K**) Myosupressin-like IR in anterior neurons and putative neurohaemal areas in the dorsal perignaglionic sheath of the synganglion. (**L**) Kinin-like IR in anterior synganglion neurons and the same neurohaemal areas as in (**K**). (**M**) Composite view of MIP-like IR in most identified dorsal and ventral neurons. (**N**) Composite confocal sections of the synganglion showing crustacean cardioactive peptide (CCAP)-like IR in all described neurons. (**O**) Tachykinin like IR in dorsal cell bodies and elaborate axonal arborizations of Pd_1SG neurons. (**P**) Allatostatin-like IR in selected dorsal and ventral neurons. (**R, S**) Allatotropin-like IR in dorsal (**E**) and ventral (**F**) sides of the synganglion. Scale bars is 50 µm (A, C-E, G-I, K-S), 20 µm (B, F, J).

Right panel: Schematic drawings of all peptidergic neurons described in the synganglion. (Šimo et al., 2009a).

Nomenclature of peptidergic neurons: The first two letters refer to the position of each neuron in a specific lobe of the synganglion: cheliceral (Ch), palpal (Pa), stomodeal (St), prothocerebral (Pc), pedal 1–4 (Pd1–4), opisthosomal (Os), preoesophageal (Pe) or postoesophageal (Po), whereas the following letters refer to its dorsal (D), ventral (V), anterior (A), posterior (P), medial (M) or lateral (L) location. Neurons projecting axons onto the surface of the putative neurohaemal perignaglionic sheath or lateral segmental organs are considered to be neurosecretory cells (NC); interneurons (IN) arborize within the synganglion, whereas neurons innervating ducts and/or acini of salivary glands are denoted as SG.

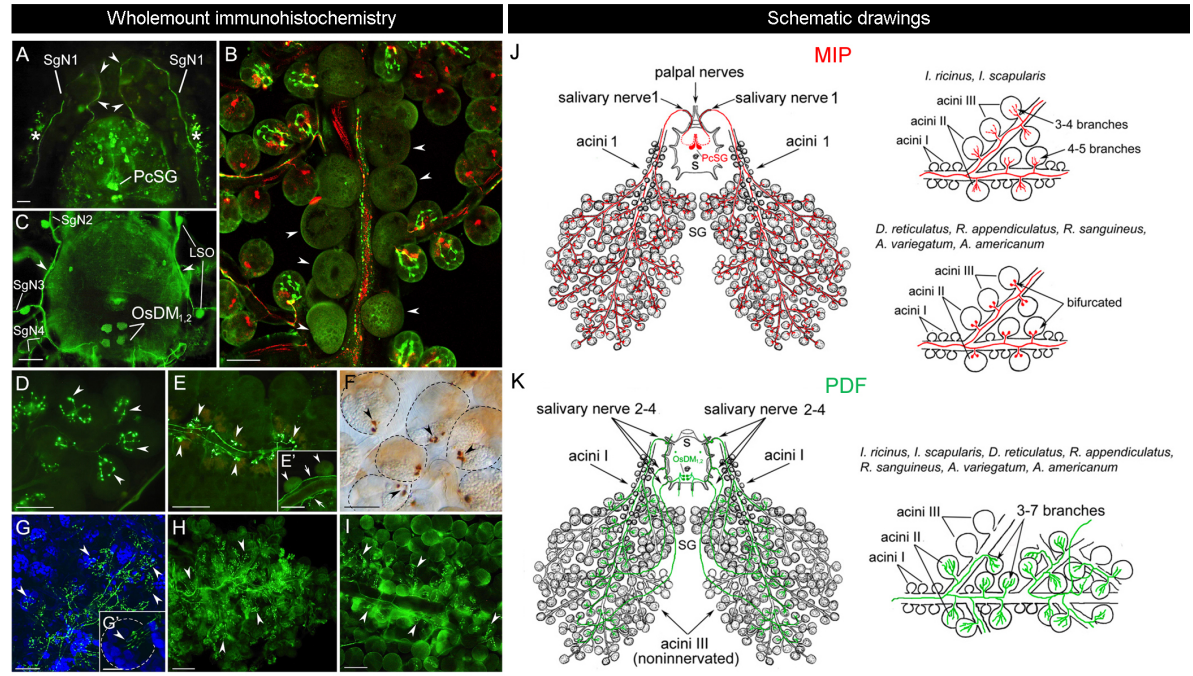


Figure 3. Left panel: Myoinhibitory peptide (MIP) and pigment dispersing factor (PDF) immunoreactivity (IR) in synganglia and salivary gland (SG) innervations in different tick species. (**A**) Pre-esophageal dorsal site of synganglion stained with MIP antibody in an unfed *I. ricinus* female. Two axons (arrowheads) from protocerebral salivary gland neurons (PcSG) excite the synganglion through the palpal nerve and salivary nerve 1 (SgN1) and innervate the SG acini II and III (asterisk). (**B**) Merged confocal z-stacks of an unfed *R. appendiculatus* female SG showing double staining with antibodies to PDF (green) and MIP (red). Note the PDF-like IR in axons containing varicosities along salivary ducts and multiple axon terminals exclusively on acini type II, whereas axons containing MIP-like IR terminate as bifurcated axon terminals on both acini types II and III. Acini I located along the main salivary duct are not innervated (arrowheads). (**C**) PDF-like-IR in the dorsal site of syngaglion in an unfed *R. appendiculatus* female. Four opistosomal neurons ($OsDM_{1,2}$) excite IR axons to the lateral nerve (lateral nerve, arrowheads) and SG nerves 2–4 (SgN2-4). Four lateral segmental organs (LSO) sitting on SgN2 and 3 were also PDF-like positive. (**D–F**) MIP-like IR (green in D and E, dark brown in F) axon terminals (arrowheads) in SG acini II and III in females from three tick species. (**D**) Unfed *I. ricinus* female.(**E**) Four-day fed *Amblyomma variegatum* larvae, (**E'**) MIP-like IR axons running along the main salivary duct (arrows) while acini type I (arrowhead) are not innervated. (**F**) Unfed *D. reticulatus* female. (**G–I**) PDF-like IR (green) innervation of SG in females from three tick species (arrowheads PDF-like IR axon terminals). (**G**) Unfed *I. scapularis* female. (**G'**) Higher magnification of type II acinus. (**H**) Unfed *I. ricinus* female. (**I**) Unfed *D. reticulatus* female. Note that in all three cases, only type II acini are innervated (arrowheads). Blue in G is DAPI staining for nuclei. Scale bars are 50 μm (for A and C) and 30 lm (for B and D–I). **Right panel:** Schematic representations of identified central neurons innervating SG. (**J**) MIP-like immunoreactive giant neurons (PcSG,red) exit through the salivary nerve I and innervate acini type II and III. Note that different shape of MIP-positive axon terminals was observed among multiple hard tick species (right schemas). (**K**) PDF-like immunoreactive neurons ($OsSG_{1,2}$, green) exit the synganglion (S) through lateral and salivary nerves 2-4 to innervate specific acini type II. Branching axon terminals contain varicosities (right schema). (Šimo et al., 2009a, 2012), For nomenclature of peptidergic neurons see legend in Figure 2.

3.2 POST-DOCTORAL/RESEARCH ASSISTANT PROFESSOR, KANSAS STATE UNIVERSITY (USA)

3.2.1 Neuropeptidergic control of tick salivary glands

The crucial point in my scientific career was the discovery of the peptidergic control of tick SG (Šimo et al., 2009a), during my PhD studies (Fig. 3). Based on this finding, I joined the arthropod molecular physiology laboratory at Kansas State University (KSU, USA) in 2007 as a post-doctoral researcher, where I continued and expanded my highly innovative research into neuropeptide signaling pathways in arthropods. Despite the fact that the laboratory was strongly insect oriented and my academic advisor had never worked in tick field before, within eight months I was able to encourage a shift of the whole lab's focus towards tick research. Here I continued and expanded my unique research into tick SG molecular physiology. I was particularly interested in elucidating further on the central discovery of my PhD thesis work, namely peptidergic SG innervations in ticks (Fig. 3). The *Ixodes scapularis* (black-legged tick) genome sequencing project based at Purdue University (Gulia-Nuss et al., 2016) and the excellent proteomics equipment (MALDI TOF/TOF) available at KSU Biotechnology/Proteomics Core Facility allowed me to identify the specific neuropeptides, and their respective genes, involved in the innervation of the tick SG to complement my previous immunohistochemical finding (Šimo et al., 2009a). During this work, in addition to MIP- positive innervations, of tick

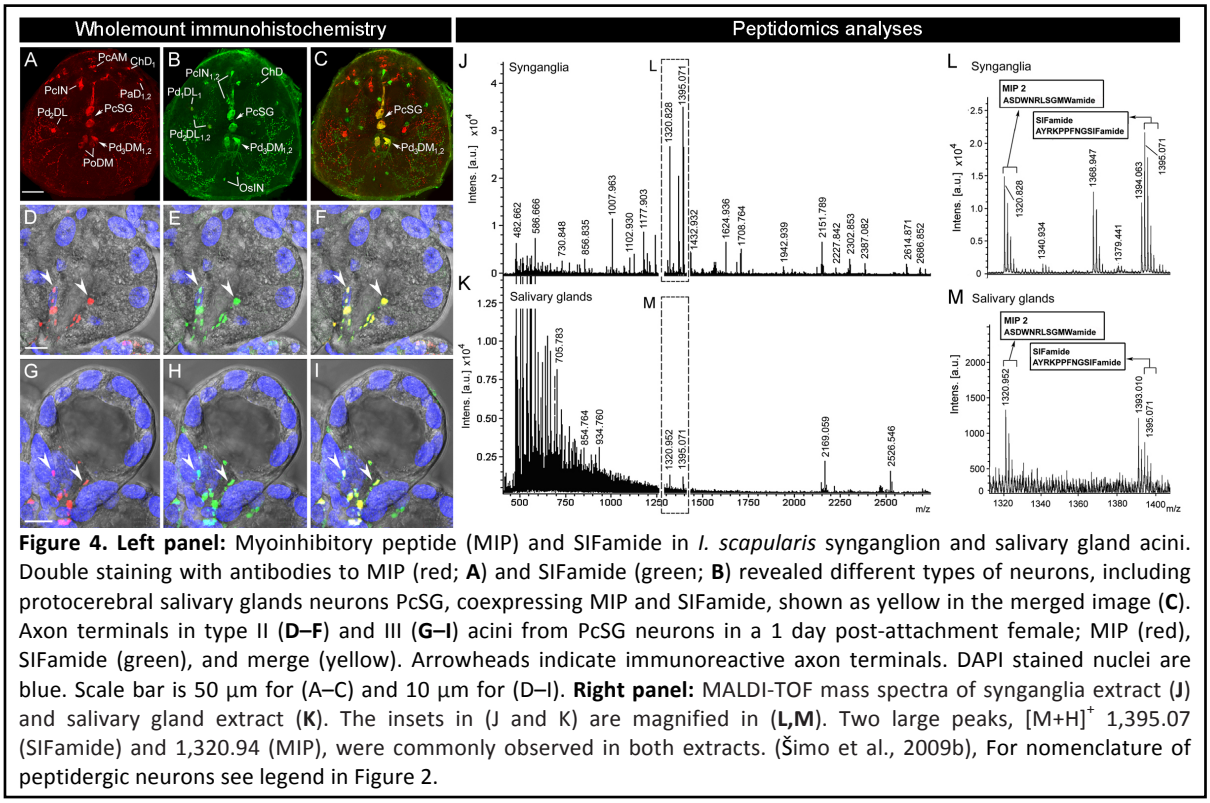


Figure 4. Left panel: Myoinhibitory peptide (MIP) and SIFamide in *I. scapularis* synganglion and salivary gland acini. Double staining with antibodies to MIP (red; **A**) and SIFamide (green; **B**) revealed different types of neurons, including protocerebral salivary glands neurons PcSG, coexpressing MIP and SIFamide, shown as yellow in the merged image (**C**). Axon terminals in type II (**D–F**) and III (**G–I**) acini from PcSG neurons in a 1 day post-attachment female; MIP (red), SIFamide (green), and merge (yellow). Arrowheads indicate immunoreactive axon terminals. DAPI stained nuclei are blue. Scale bar is 50 µm for (A–C) and 10 µm for (D–I). **Right panel:** MALDI-TOF mass spectra of synganglia extract (**J**) and salivary gland extract (**K**). The insets in (J and K) are magnified in (**L,M**). Two large peaks, $[M+H]^+$ 1,395.07 (SIFamide) and 1,320.94 (MIP), were commonly observed in both extracts. (Šimo et al., 2009b), For nomenclature of peptidergic neurons see legend in Figure 2.

SG, I found another neuropeptide, SIFamide, to be co-expressed with MIP in the SG innervating giant neurons PcSG and their axons reaching basal parts of type II and III SG acini of *I. scapularis* (Fig. 4A-I, 5A-C). Based on these findings we predicted that neuropeptides produced by synganglion and delivered via axonal projections to SG acini could be detected in MALDI peaks that were present in both the extracts of the synganglia and the SG. Indeed, two MALDI peaks were present in both extracts: 1,322 and 1,396 Da (Fig. 4J-M). Tandem mass spectrometry of these peaks provided partial sequences and amino acid compositions. Combining this information with data from the *I. scapularis* sequence database yielded sequences corresponding to MIP2 (ASDWNRLSGMWamide: 1,321.6106 Da) and SIFamide (AYRKPPFNGSIFamide: 1,395.7532 Da) as the best matches (Fig. 4 J-M). These results were published in *Journal of Comparative Neurology*, which also included a ***front page illustration*** for the issue displaying the work (Šimo et al., 2009b) (see on the right site of this paragraph). Furthermore, this data was used as the basis for an NIH grant, R21-AI081136-01 (PI: Yoonseong Park) titled "*Neuropeptidergic Control of the Salivary Gland in the Black-legged Tick, I. scapularis*" (total of 250 000 dollars) granted for the laboratory. I produced all preliminary and final data of this grant funding. Furthermore, I molecularly identified both MIP and SIFamide receptors (MIP-R, SIFa-R respectively) being expressed in *Ixodes* SG. Furthermore we generates affinity purified antibodies against both receptors to visualise them in the *Ixodes* SG using immunohistochemistry approach (Fig. 5 D-I)(Šimo et al., 2013). Here I was able to visualise only the SIFa-R while MIP-R receptor failed in the immunostaining. In either case the anti-SIFa-R antibody showed positive reaction in close proximity to the SIFamide-positive axon terminals reaching the basal part of both type II and III acini (Fig. 5D-I). Additionally, I succeeded to functionally characterise both receptors in our mammalian cell line expression system. Particularly the activities of both receptors were tested in Chinese hamster ovary cells (CHO cells) for triggering the intracellular calcium mobilisation downstream pathway. Both receptors show nanomolar sensitivity to their authentic ligands (Fig. 5 J-M) representing the first neuropeptide receptors functional identification in ticks. This results were published in the *Insect Biochemistry and Molecular Biology* (Šimo et al., 2013). In summary these two publications (Šimo et al., 2009b, 2013) proofed, that mature MIP and SIFamide neuropeptides are delivered via the axonal projections to the SG acini II and III for activation of their transmembrane receptors expressed on the base of the basal acinar cells (Fig. 5). Although this findings were completely new and very informative, the real functions of the examined neuropeptides innervating the tick SG remained elusive. At this point we speculated that the associations of neuropeptidergic axon terminals as well as their targeted receptors with luminal neck region of the acinus, indicates the neuropeptidergic control of the acinar valve, the structure regulating the expulsion of saliva to the associated ducts (Fig. 5 D-I).

Because of a successful post-doc speed–up start at KSU, I have then been promoted to Research Assistant Professor by KSU provost. After this, I immediately became a mentor for the students and other post-docs in hosted laboratory. My mentoring included broad variety of techniques related to tick molecular physiology research I am familiar with such as: immunohistochemistry, histology, *in situ* hybridization, real-time PCR, peptidomics, bioinformatics, RNAi, tick feeding, confocal imaging, G-protein coupled receptor (GPCR) functional assay, cell culture and physiology.

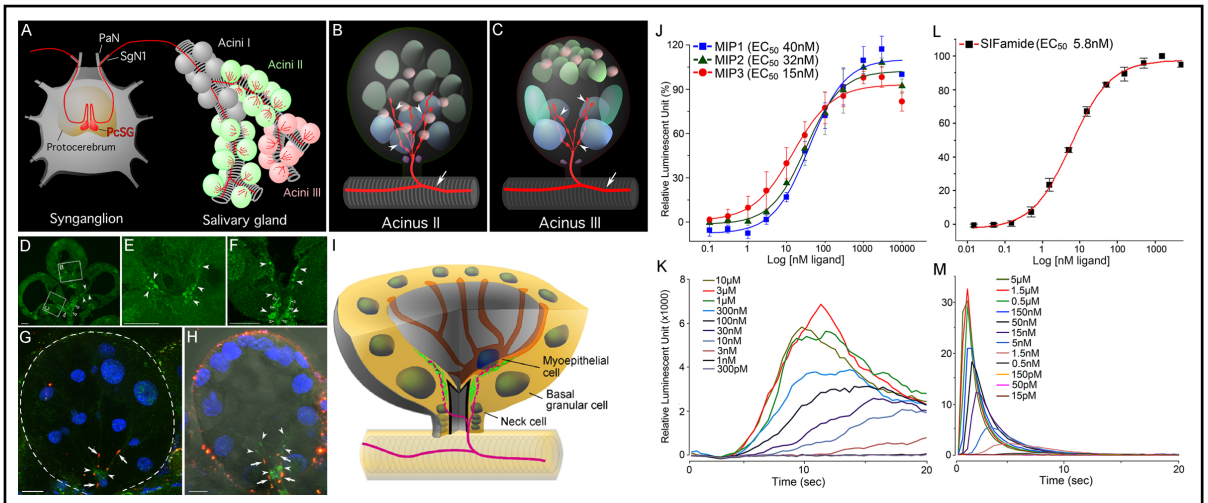


Figure 5. (**A**) Simplified schematic diagram showing the peptidergic axonal projection (MIP and SIFamide) from the PcSG neuron to the salivary glands. Different colors of nuclei in **B** and **C** indicate different types of cells based on their size and shape in the confocal image. Four or five axonal branches in acini II and three or four branches in acini III are in contact with the basal cells that have large, round nuclei.(**D-I**) Immunohistochemical analysis of the SIFamide receptor (SIFa-R, green, arrowhead) in tick salivary gland acini. (**D-F**) Type III acini was isolated from four-day-fed females and sectioned. The empty arrow heads indicate the salivary duct in D and the acinar duct in F which were obscured by the autofluorescence of the cuticular lining of the duct. (**G, H**) The SIFamide neuropeptide (red, arrow) and its receptor (SIFa-R, green, arrow head) in a whole-mount staining of acini type II of partially fed *Ixodes* female. Note that the neuropeptidergic signal (red) is closely associated with the basally localized SIFa-R (green). (**I**) Simplified diagram of the acini type III basal region. The nuclei of different cells are shown as round circles. SIFamide peptidergic innervations (magenta) are closely associated with SIFa-R receptors (green) as depicted. Three different types of cells in the basal region are annotated, with the cell boundary indicated only for the mypepithelial cell, as described in Krolak et al. (1982). Scale bar indicates 10 μm. (**J-M**) Aequorin reporter assays in Chinese hamster ovary cells (CHO) cells that were transfected with either myoinhibitory peptide receptor (MIP-R; **J, K**) or SIFamide receptor (**L, M**). The results are the cellular responses observed after ligand-mediated calcium mobilization. (**J**) The dose-response curves for MIP-R to the MIP ligands (i.e., MIPs 1, 2, and 3) in *I. scapularis*. (**L**) The dosee response curve for SIFa-R to *I. scapularis* SIFamide. (**K**) Representative responses of the cells to MIP-R when treated with different doses of the MIP ligands (MIP2 in this case). (**M**) Representative responses of the cells to SIFa-R when treated with different doses of SIFamide. The bars in A and B indicate the standard error for a minimum of three replicates. (Šimo et al., 2013)

3.2.2 Signaling pathways for tick salivary secretion

My previous achievements in the field of peptidergic innervations (Šimo et al., 2009a, 2009b, 2012, 2013) of tick SG confirmed the previous reports describing nerves arising from the synganglion to SG and to date there has been no evidence that this tissue is hormonally controlled (Kaufman, 1976, 1978; Kaufman and Harris, 1983; Sauer et al., 1995).

Tick SG secretion is a complex dynamic process involving multiple biological pathways, and over the last four decades a number of components that regulate tick SG secretion have been discovered (Bowman et al., 2008; Bowman and Sauer, 2004; Kaufman, 1976, 1978, 1977; Kaufman and Harris, 1983). Among these, catecholamines have been shown to be powerful activators of SG fluid secretion in both *in vivo* and *in vitro* assays (Kaufman and Harris, 1983; McSwain et al., 1992; Sauer et al., 2000). Specifically dopamine-mediated tick salivary secretion has been widely used as an experimental tool to study salivary secretion in multiple hard tick species. For over three decades, researcher believed that in the SGs dopamine is released from the synaptic boutons arising from dopaminergic neurons located in the tick central nervous system (Bowman and Sauer, 2004; Sauer et al., 2000; Sonenshine, 1991). While earlier studies of SG physiology were largely based on pharmacological approaches, our achievements in the post-genomic era have led to breakthroughs in the

molecular understanding of the processes underlying regulation of this important organ (Bowman and Sauer, 2004; Kaufman, 1976, 1978, 1977; Kaufman and Harris, 1983). Specifically, using immunohistochemistry and electrochemical approaches we revised the paradigm of synaptic dopamine in tick SG and proved that type II and III acini are the major pool of dopamine (Šimo et al., 2011, Koči et al., 2014). Thus it appears that dopamine acts as an autocrine/paracrine signal in tick SG (Fig. 6. A-H). Interestingly we detected the immnureactive dopamine in the basal cells of acini type II and III in the early phase of *Ixodes* feeding (Fig. 6A-F') (Šimo et al., 2011). Furthermore our electrochemical analyses detected free dopamine, conjugated dopamine and dopamine sulfate in the SG during entire course of *Ixodes* female feeding (Fig. 6G, H) (Koči et al., 2014). Here it is important to highlight that no dopaminergic axons in SG were spotted in our immunohistochemistry experiments.

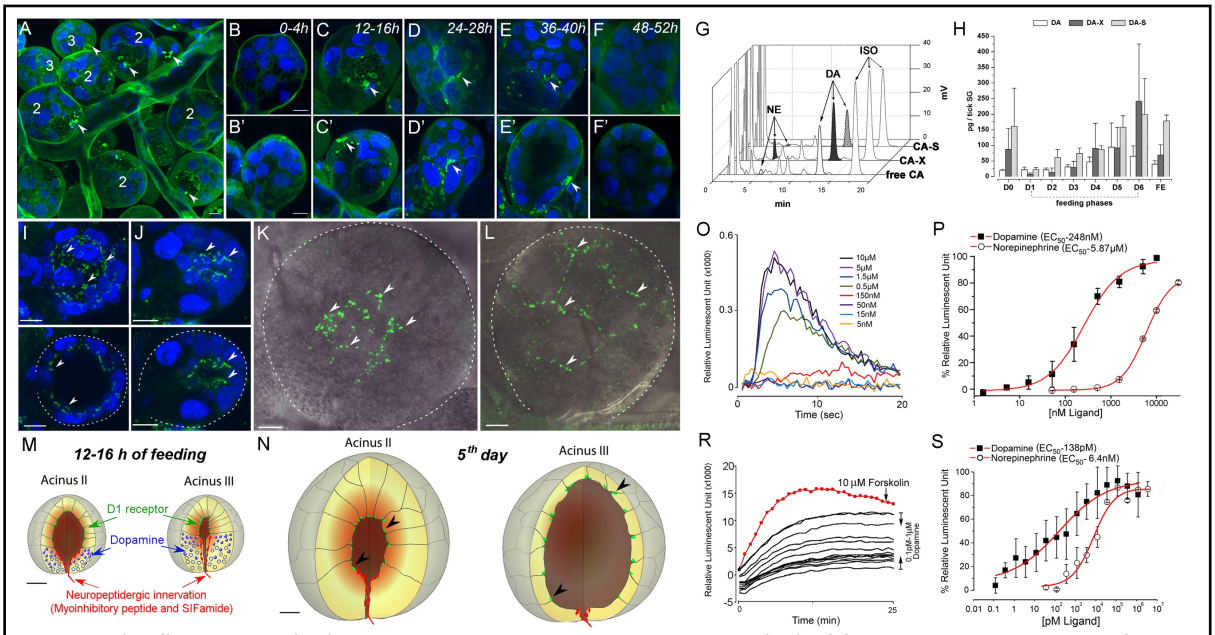


Figure 6. (**A-F'**) Dopamine (DA) immunoreactivities in the salivary glands (SG) of female ticks during various feeding phases. (**A**) A region of SG with clustered acini II (labeled 2) and III (labeled 3) at 12–16 h after attachment. (**B** to **F**) Acini II at 0–52 h after attachment to the host and (**B'**to **F'**) acini III at the same feeding phases. Note that positive staining (green marked with arrowheads) was detected in both acini II and III in the vesicles and their surrounding regions, but only at 12–40 h post-attachment. The blue color shows nuclei stained with DAPI. Scale bars are 10 µm. (**G**) HPLC–ECD chromatograms of the free catecholamines (free CA), hydrolysed catecholamines (CA-X) and sulphohydrolysed catecholamines (CA-S) extracted from SG of 5 day-fed female *I. scapularis* tick. (**H**) Feeding phase-specific quantification of dopamine and its conjugates in tick salivary glands. D1-D6 are days of feedings, FE – fully engorged. (**I-L**) Immunohistochemistry for D1 dopamine receptor (green, arrowheads) in the *Ixodes* SG. (**I**) Whole acinus II of unfed female (upper panel) and a selected thin optical layer (lower panel) and (**J**) whole acinus III of an unfed female (upper panel) and a selected thin optical layer (lower panel) demonstrating the apical location of the D1 receptor immunoreactivities (green arrows). Blue is DAPI staining for nuclei. (**K**) Acini II and (**L**) III of a female at the 5th day post-attachment. Scale bars are equally for 10 µm. (**M, N**) Schematic diagram showing the D1 (green), DA (blue), and neuropeptidergic innervation (red) in acini II and acini III of the 12–16 hour fed female (**M**) and 5th day postattachment in a female (**N**). Arrowheads indicate the scattered patches of D1 receptor reaction on the luminal side of the acini. (**O-S**) Reporter assays for D1 receptor showing DA-activated calcium mobilization and cAMP elevation. (**O**) Luminescent reporter calcium assay showing typical responses to varying doses (5 nM to 10 µM) of DA. (**P**) Dose–response curves of the Chinese hamster ovary cells (CHO) cells transfected with the D1 and aequorin constructs and treated with DA and norepinephrine. (**R**) Luminescent reporter cAMP assay (GloSensor) showing typical responses to varying doses (0.1 pM to 1 µM) of DA. (**S**) Dose–response curves of the HEK cells transfected with the D1 and GloSensor constructs for DA and norepinephrine. Bars indicate standard errors for a minimum of four replicated plates. A-N and O-S is from Šimo et al., (2011), G and H are from Koči et al., 2014

A simple *in vitro* experiment using isolated SG of partially fed *Ixodes* female showed that when SG are exposed to dopamine two specific actions of acini III can be observed (Fig. 7): 1. Pumping, where acini show periodical filling with fluids followed by acinar content expulsion and, 2. Gating, where acini are filled by fluids while the is not acinar content release. Based on this observation we predicted that these two actions may by controlled by dopamine-mediated downstream signaling pathways via the representative receptors (Kim et al., 2014).

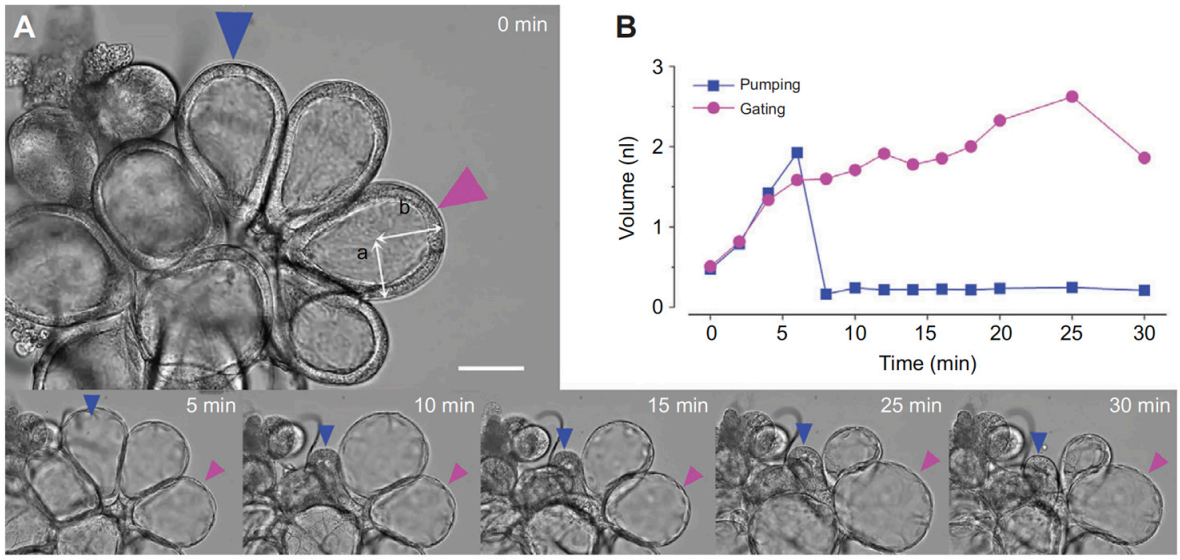


Figure 7. Dynamic responses of individual salivary gland type III acini to dopamine treatment. (**A**) An example indicating the changes of *I. scapularis* salivary gland acini recorded by a video camera for 30 min after 10 µmol^{-1} dopamine treatment. Arrowheads indicate two randomly selected acini for measuring the volume change. The lengths a and b demonstrate the axes that were used for calculation of the volume. Colors of arrowheads correspond to the line colors in B. (**B**) Each line indicates changes in individual acini volume to dopamine treatment for 30 min. Blue and purple lines indicate pumping and gating, respectively. Scale bar, 50 µm. Video available in original article. (Kim et al., 2014)

Although dopamine is known as the most potent stimulator of tick SG secretion, despite the enormous efforts of multiple laboratories around the world in last three decades, no dopamine receptor had been identified in ticks. Using *I. scapularis* genome database (Gulia-Nuss et al., 2016) I have, for the first time, molecularly characterized two different dopamine receptors in *Ixodes* tick (Šimo et al., 2014, 2011). Both these receptors have been found to be expressed in the SGs of *I. scapularis*: the D1 dopamine receptor and the invertebrate-specific D1-like dopamine receptor (InvD1L). Immunohistochemistry targeting the intracellular C-terminals of both receptors revealed the presence in both type II and III acini of *Ixodes* SG. In particular, the immunoreaction of D1 receptor was spotted in scattered patches in the cell junctions on the luminal surface of type II and III acini (Fig. 6I-N), while InvD1L has been evidenced in the axon terminals (with unknown origin) lining the acinar valve and lumen in the same type of acini (Fig. 8 A-L). Thus, here, we suggested that secretory activities via SG type II and III acini are controlled via both, epithelial (D1 dopamine receptor) and axonal (InvD1L) dopamine receptor. Here an importance has been placed on the InvD1L-positive axon terminals within the acini that were found to be in close proximity to the myoepithelial cell that lines the acinar lumen (Fig. 8A-L, also see Fig. 14, 16N-Z and 17). A single adlumenal myoepithelial cell (Fig. 8A-L, 15A, 17) within each of the acinus type II and III has been described by multiple authors and suggested for its contractile like properties,

likely helping to release acinar contents to the associate ducts (Coons et al., 1994; Krolak et al., 1982).

Subsequently pharmacological profiling of these receptors in a heterologous expression system has revealed that activation of the D1 receptor triggers both intracellular calcium mobilization and the cAMP-dependent downstream pathways (Fig. 6 O-S), while activation of the InvD1L resulted exclusively in mobilization of intracellular calcium (Fig. 8M-P) (Šimo et al., 2014, 2011). The in *vitro* pharmacological tools allowed us to select specific agonists/antagonists for selective activation or blocking either of these receptors in live type II and III acini in our *in vitro* assays. Based on the *in vitro* pharmacological experiment of two different dopamine receptors we proposed a distinct physiological role for each of these receptors; namely, the epithelial D1 receptor regulating the inward transport of fluid into the acini, while the axonal InvD1L receptor controlling the expulsion of the acinar context into the connecting ducts, presumably via the contractile properties of the myoepithelial cell (Kim et al., 2014).

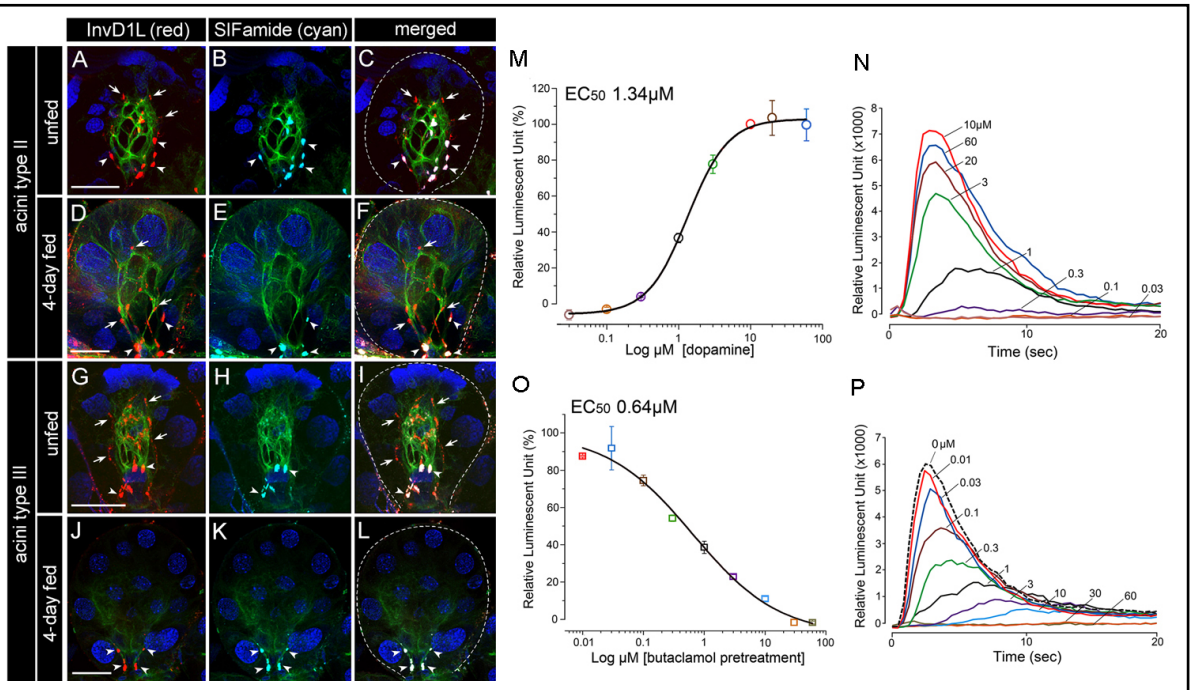
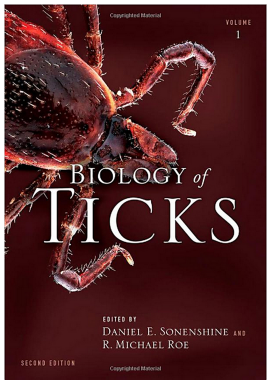


Figure 8. (**A-L**) Immunohistochemistry for InvD1L receptor (red, **A,D,G,J**), SIFamide neuropeptide (cyan, **B,E,H,K**), and b-tubulin (green). White color in the merged image (**C,F,I,L**) indicates colocalization (arrowheads) of InvD1L receptor and SIFamide neuropeptide. Blue color represents DAPI staining for nuclei. Scale bars are 20 µm. Longer axon-like processes only for InvD1L receptor (red) are marked by arrows. Note that the basally located axon terminals stained for both InvD1L (red) and SIFamide (cyan) are marked by arrowheads. In later study (please see the section XXX) we confirmed that these basal axon terminals are specific only for SIFamide, and not InvD1L. In presented figure it seems that InvD1L basal axons are a non-specific reaction. (**M-P**) Bioluminescent aequorin reporter assays for InvD1L receptor expressed in Chinese hamster ovary cells (CHO). (**M**) Dose–response curve of InvD1L receptor to various doses of dopamine (DA). (**N**) Representative luminescence responses of InvD1L when treated with different doses of DA (30 nM to 60 µM). (**O**) Dose– response curves of the antagonistic activity of (+)butaclamol on the DA-mediated (10 µM) InvD1L receptor activity (**P**) Representative luminescence responses of InvD1L to 10 µM DA after preincubation with different concentrations of and dopamine receptor antagonist (+)butaclamol for 15 minutes. The bars in M and O indicate the standard error for three replicates. (Šimo et al., 2014)



Furthermore, my discovered model of catecholamines and neuropeptides signaling in tick SG served as the scientific basis for obtaining additional NIH grant funding through R01-AI090062 (PI: Yoonseong Park) "*Signaling Pathways for Tick Salivary Secretion*" (total of 1 million dollars). This scientific outcome has led to a completely new understanding of the system of tick SG control, and the finding has potential application for disrupting the lifecycle or pathogens transmission of any tick species throughout the world. The research I have been conducting in the field of tick molecular neurophysiology is highly valued in the tick community, and this is why I was invited to be a senior leading author for a second edition of book chapter, *The Nervous and Sensory Systems: Structure, Function, Proteomics and Genomics; Biology of Ticks Volume 1*, (Šimo et al., 2014) (see on right site of this paragraph) .The book, which has been published 20 years after the first edition, is the most valuated summary of tick research and only the researchers recognized as the leaders in particular topic were invited to write a chapter. The opportunity to summarize the discoveries I made in the field of tick neurobiology in this publication, was definitely the highlight of my scientific career for years to come.

3.2.3 Neuropeptidergic control of the hindgut in ixodid tick

Beside the tick SG that a play a pivotal role in tick body osmoregulation, another important excretory/osmoregulatory organ playing a significant physiological role in maintaining homeostasis and waste elimination is the tick's hindgut. The hindgut is comprised of Malpighian tubules, the rectal sac and the short anal canal (Sonenshine, 2014). Although the regulation of diuresis has been extensively studied in insects (Park, 2012), little is known about the mechanisms in ticks.

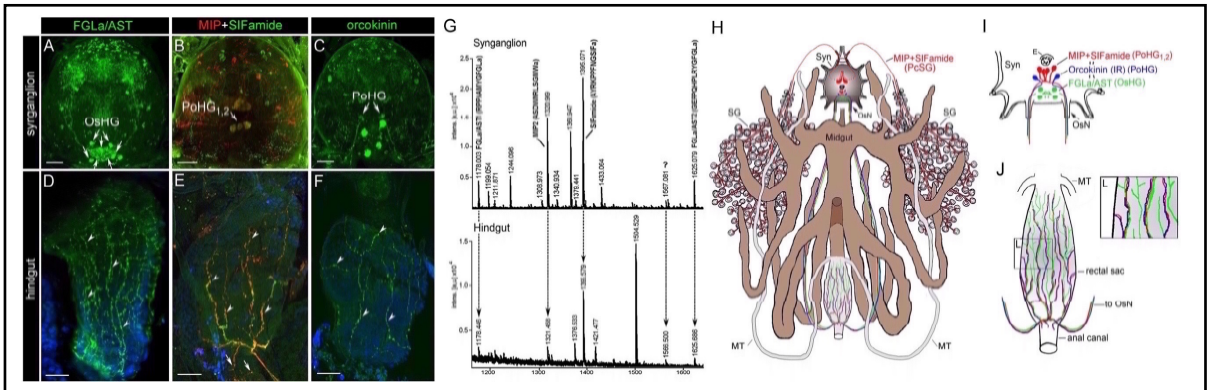


Figure 9. (A-F) Immunoreactivity of neuropeptides in unfed *I. scapularis* female synganglia and hindguts. (**A–C**) Immunoreactivity of neuropeptides in synganglia. Arrows indicate neuronal cells innervating the hindgut. The yellow colour in B represents the colocalization of myoinhibitory peptide (MIP) (red) and SIFamide (green). (**D**) FGLamide related allatostatin immunoreactivity innervation of the rectal sac (arrowheads). (**E**) Colocalization of myoinhibitory peptide and SIFamide in the innervation of the rectal sac (arrowheads) and the anal canal (arrows). (**F**) Orcokinin immunoreactivity innervation of the rectal sac (arrowheads). PoHG, postoesophageal hindgut neurons. Blue colour represents DAPI staining for nuclei. Scale bars are 20 µm. (**G**) MALDI spectra of Ixodes scapularis synganglia (upper panel) and hindgut extract (lower panel). The peaks are annotated based on the [M+H]+ with the peptide sequences in parentheses. Simplified diagrams showing peptidergic innervation of Ixodes scapularis female hindgut. (**H**) Complex view of synganglion (Syn) neurons innervating the hindgut and salivary glands (SG). (**I**) Magnified view of dorsal-postoesophageal region of the synganglion with a focus on putative peptidergic neurons innervating the hindgut described in this study. Note that immunoreactive axonal projections from all detected neurons exit the synganglion via the opisthosomal nerves (OsN) and reach the surface of the rectal sac and the anal canal as shown in (**J**). (Šimo and Park 2014)

During feeding, up to 74% of potassium and about 25% of water is excreted via tick's hindgut (Kaufman and Phillips, 1973). The feces, or wastes, from the midgut, consists of hematin, other nutrients and undigested hemoglobin. These fecal wastes are passed to the rectal sac.

During my screening of multiple neuropeptides immunoreactivities in tick body I discovered that parts of the tick alimentary canal are under the neuronal command. Specifically, I have described for the first time the connection of specific neuronal cells within the tick synganglion with the hindgut (Fig. 9) (Šimo and Park, 2014). I revealed that in *I. scapularis*, a rich neuropeptidergic axonal network arising from specific neurons in the tick synganglion arborize on the surface of the tick rectal sac and anal canal (Fig. 9A-F, H-J). Distinct immunoreactive axons reacting with four different neuropeptides were described: FGLamide related allatostatin, MIP and SIFamide (both co-expressed in the same axonal projections) and orcokinin (Fig. 9A-F).

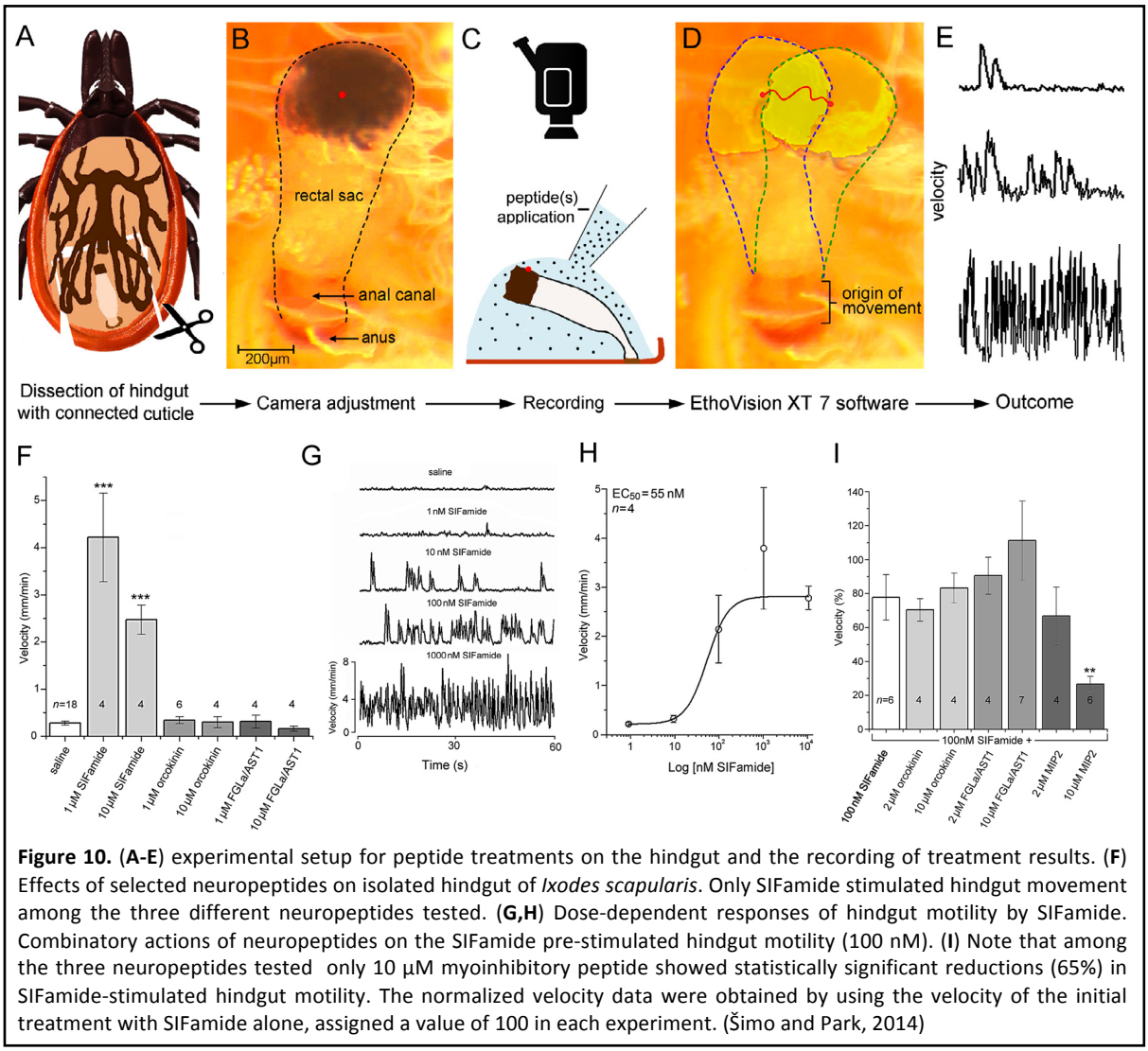


Figure 10. (**A-E**) experimental setup for peptide treatments on the hindgut and the recording of treatment results. (**F**) Effects of selected neuropeptides on isolated hindgut of *Ixodes scapularis*. Only SIFamide stimulated hindgut movement among the three different neuropeptides tested. (**G,H**) Dose-dependent responses of hindgut motility by SIFamide. Combinatory actions of neuropeptides on the SIFamide pre-stimulated hindgut motility (100 nM). (**I**) Note that among the three neuropeptides tested only 10 µM myoinhibitory peptide showed statistically significant reductions (65%) in SIFamide-stimulated hindgut motility. The normalized velocity data were obtained by using the velocity of the initial treatment with SIFamide alone, assigned a value of 100 in each experiment. (Šimo and Park, 2014)

Furthermore I employed a peptidomic approach to confirm the presence of earlier three neuropeptide in both synganglia and hindgut extracts, supporting our immunohistochemistry approaches (Fig. 9 G). The tissue specific PCR revealed that receptors for MIP, SIFamide and FGLamide related allatostatin are expressed in the *Ixodes* hindgut,

while no orcokinin receptor has been identified in any invertebrates yet. These data suggest that tandem action of these neuropeptides and their cognate receptors, may regulate activities of this tissue (Šimo and Park, 2014). The discovery of peptidergic control of tick hindgut let us establish an *in vitro* physiological experiment (Fig. 10A-E) where, for the first time, a strong myostimulatory effects of SIFamide on hindgut motility has been observed, whereas MIP antagonized these actions (Fig. 10F-I). The SIFamide stimulated the hindgut motility in dose-depended manner (Fig. 10F-H), while a slightly higher concertation of MIP was needed to inhibit the actions of SIFamide (Fig. 10I).

In either case the bioactivity of SIFamide/MIP on the dissected hindgut demonstrated the first direct evidence for specific role of these tick neuropeptides. Along with their proposed functions in the control of SG (Fig. 4,5), these two peptides are hypothesized to function as important regulatory factors of both primary osmoregulatory organs in ticks (Kim et al., 2019). The results have been published in the *International Journal for Parasitology* and *Cell and Tissue research* (Roller et al., 2015; Šimo and Park, 2014) where for the first time the evidence of peptidergic control of tick hindgut has been shown. Both publications were published with the ***front-cover page*** of the representative issue and the first work was also selected as ***editors' choice article***, and I was the first and the co-corresponding author of this manuscript.

3.2.4 *Ixodes scapularis* genome project

In 2009, I was invited to join the North-American tick species *I. scapularis* genome sequencing consortium. The first tick genome published by this consortium described the nuclear genome of the *I. scapularis* ticks (Gulia-Nuss et al., 2016). This tick species is known as a primary vector of pathogenic agents causing a Lyme disease in North America (Walker, 1998). The large genome of this species (2.1 Gbp) accumulate a repetitive DNA and new lineages of retro-transposons. Fluorescent *in situ* hybridization (FISH)-based physical mapping was used to develop a karyotype and physical map (Fig.11 A-F). The *I. scapularis* genome possesses 26 acrocentric autosomes and two sex chromosomes. The genome-scale quantitative molecular species phylogeny, confirms the position of *Ixodes* genome (Chelicerata) as basal to insects and Crustacean (Fig. 11H). Quantification of shared intron positions (Fig.11 I) and lengths (Fig. 11 J) among orthologs reveals that *I. scapularis* shares greater than 10 times more intron positions exclusively with the non-arthropod species compared with the crustacean *Daphnia pulex*. The work has been published in 2016 in prestigious *Nature Communications* (Gulia-Nuss et al., 2016) when I was already an INRAE researcher working in UMR-BIPAR (France).

Due my expertise in tick neurobiology my particular involvement in this project was the annotation of neuropeptides and peptide hormones genes. The neuropeptide signaling system is believed to be of ancient lineage and tick neuropeptides are closely related to those identified in other invertebrates such as insects and crustaceans. Most tick neuropeptides have clear counterparts in other invertebrate species (Nässel, 2009) and my *in silico*

homology searches revealed 45 putative neuropeptides genes in *I. scapularis* (Fig. 12A) (Gulia-Nuss et al., 2016; Šimo et al., 2014).

Among those 34 genes were comparable to those found in other invertebrates (Fig. 12A). Nineteen of the 34 neuropeptide genes are also present in the EST dataset, and the presence of 18 mature peptide sequences was confirmed via MALDI TOF/TOF analyses (Neupert et al., 2009; Šimo et al., 2014). A number of putative novel neuropeptide genes (11 in total) were identified based on the repeated mature peptide-like motif sequences separated by cleavage sites (Fig. 12A). The total number of identified neuropeptide genes was lower than the number generally found in other model arthropods likely due to the difficulties experienced in obtaining the complete sequence and assembly of this large *Ixodes* genome.

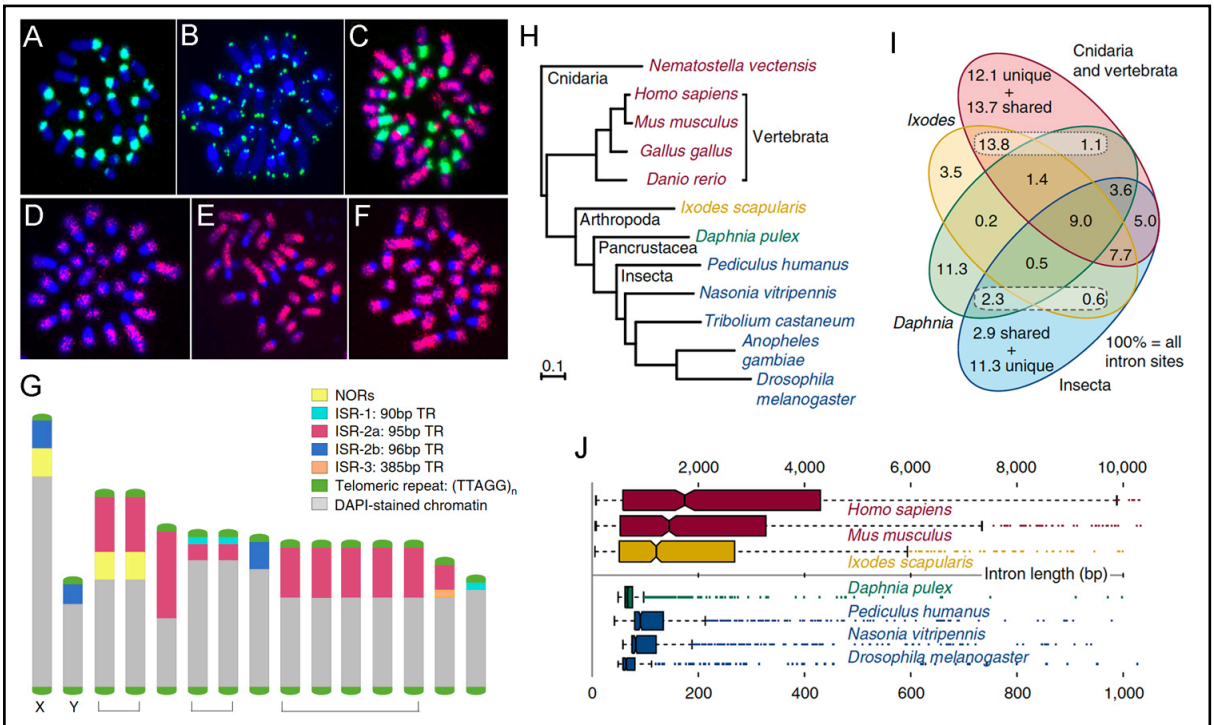


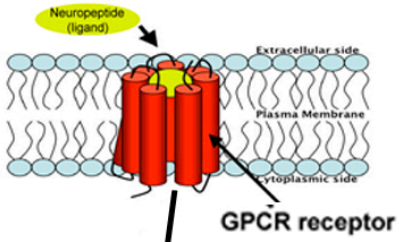
Figure 11. (**A-G**) Organization of DNA on the *I. scapularis* chromosomes. Families of tandem repeats (TRs) comprise approximately 40% of the genome and were localized by fluorescent in situ hybridization (FISH) to ISE18 cell line mitotic chromosome spreads. (**A**) Representative FISH image of Cot-1 DNA (green) at the heterochromatic terminal region of the DAPI-stained chromosomes (blue), presumed to represent the centromere. (**B**) Representative FISH of a telomeric repeat probe (TTAGG)n. (**C**) Representative FISH of a BAC clone (BAC ID: 192414) in red and the ISR-2a 95 bp tandem repeat in green. BAC clone hybridization signals are dispersed throughout the presumed euchromatic regions of the DAPI-stained chromosomes (blue). (**D-F**) FISH using probes from clones in a small-insert gDNA library containing tandem repeats; Clone O-21 (d); Clone B-20 (e); Clone B-01 (**F**). Note that the hybridization signals (red) are dispersed among the presumed euchromatic regions of the DAPI-stained chromosomes (blue) and not at the heterochromatic termini thought to represent the centromeres. (**G**) Ideogram showing the relative arrangement of tandemly repetitive DNA based on FISH to the presumed acro- or telocentric chromosomes. The 13 autosomes and the X and Y sex determining chromosomes are shown. Brackets indicate groups of chromosomes sharing similar hybridization patterns. The individual chromosomes within these groups could not be distinguished based on relative size or distribution of tandemly repetitive DNA. Chromosomes are drawn to scale based on the representative example. Variability in Continued the relative sizes of ISE18 chromosomes among different chromosome spreads prevented development of a standard karyotype where chromosomes are assigned numbers based on size and FISH marker distribution. (**H-J**) Molecular and intron evolution of *I. scapularis* orthologs. (**H**) The species phylogeny computed from the concatenated alignment of single-copy orthologous protein-coding genes confirms the position of the Subphylum Chelicerata at the base of the arthropod radiation, an outgroup to the clade Pancrustacea that contains crustaceans and hexapods. (**I**) Quantification of the proportions of shared and unique intron positions from well-aligned regions of universal orthologs reveals that, compared with the crustacean, *Daphnia pulex*, *I. scapularis* shares more than 10 times as many introns exclusively with at least one of the five outgroup species (from Cnidaria and Vertebrata) (dotted box, 13.8% versus

Figure 11 ***continued:*** 1.1%). Conversely, *D. pulex* has more intron positions exclusively in common with the representative insects (dashed box, 2.3% versus 0.6%). (**J**) *I. scapularis* intron lengths are more similar to those of introns from orthologous genes in the vertebrates Homo sapiens and Mus musculus, and are an order of magnitude longer than introns from the pancrustacean species analysed. The intron length distributions are shown for ancient introns found in both *I. scapularis* and *D. pulex* and at least one of the five outgroup species and at least one insect; boxplots indicate medians, first and third quartiles, and whiskers. Gulia-Nuss et al., 2016)

A

Name	Supercontig	Coordinates	Computed annotation
Achatin-like (GFGE)	DS940350	23019..23117	NA
AKH (ACP)	DS968442	24720..24893	NA
Allatostatin A	DS971562	340315..339812	ISCW022939
Allatostatin B (myoinhibitory peptide)	DS704057	214860..217973	ISCW017595
Allatostatin C	DS617680	26756..26517	ISCW001803
Allatosattin CC	DS614450	94160..93978	ISCW001408
Allatotropin	DS723986	69897..74019	ISCW017791
Vasopressin/Oxytocin (inotocin)**	DS955335	731..757	NA
	DS655913	50489..50686	NA
Bursicon alpha	DS725348	327154..329168	ISCW004617
Bursicon beta	DS725348	334706..336760	ISCW004618
CCAP	DS863512	155818..156096	ISCW010619
CCHamide-1**	DS920188	4270..1925	ISCW013057
	DS721341	944..1070	
Corazonin	DS968442	4830..8114	ISCW014429
Calcitonin-like diuretic hormone 34a	DS849364	213812..229510	ISCW020490
Calcitonin-like diuretic hormone 34b	DS833812	290964..308120	ISCW009341
Corticotropin-releasing factor-related diuretic hormone DH61***	DS951787	1112..1534	ISCW007845
	DS793410	18053..18115	
Eclosion hormone	DS652454	187087..184932	ISCW001941
EFLamide	DS945230	55463-66354	ISCW014582
Glycoprotein A2***	DS850534	41653..41751	NCBI prediction
	DS669550	1333..1046	
	DS957846	12481..12573	
Glycoprotein B5	DS860962	49721..66736	ISCW010926
Insulin like peptide (ILP4)	DS687889	56659..72302	ISCW002549
Ion transport peptide	DS934076	108540..97467	ISCW023228
Kinin	DS680282	583..1410	ISCW024200*
Neuroparsin	DS781496	23994..25192	NA
Orcokinin	DS860349	8710..8450	ISCW010518*
Proctolin	DS752645	23044..97988	ISCW005701*
PTTH like (Trunk)	DS624571	79215..96811	ISCW001809
Pyrokinin	DS798279	55372..56853	ISCW019582
RYamide	DS762742	10487...40630	ISCW005825
SIFamide	DS939604	10166..18491	ISCW022950
Short neuropeptide F**	DS682464	1..104	ISCW007409
	DS800964	20299..20852	
Sulfakinin	DS674693	50707..50498	NA
Tachykinin**	DS805407	18901..16589	ISCW008383
	DS714254	229..80	
Trissin	DS706258	980-1054	
Novel putative neuropeptides genes			
FLVamide	DS925401	117227..115863	NA
GTVamide-1**	DS641015	1037..1	NA
	DS726073	1037..1	NA
	DS871441	73..5	
GTVamide-2	DS873396	117488..117156	NA
IRLamide	DS963481	1316..567	NA
LHFamide	DS918990	378863..381517	ISCW012656
LHFa/AVFamide***	DS918990	305627..305271	ISCW000205
	DS647107	913..1149	
LRFamide	DS810236	227458..231328	ISCW019773*
PWGamide	DS68028	715..1383	ISCW02420
QFTa/QFAa/QLTamide	DS810352	3..1431	NA
QFAa/ HFAa/QLTamide **	DS799148	921..19	NA
	DS699187	1175..1	NA
QFAa/QVKamide	DS658524	979..2	NA

B



receptors for	no. of receptor genes: Ixodes	no. of receptor genes: other arthropods
ACP	6	1
Ast-A	4	1-2
Ast-B	2	1
Capa	3	1
Corazonin	2	1
DH (CRF-like)	5	1-2
DH (Calcitonin-like)	5	1-2
Inotocin	3	1
Kinin	4	1
PDF	3	1
sNPF	2	1
Sulfakinin	7	1-2
Tachykinin	10	1-2
Trissin	2	1

Figure 12. (**A**) Neuropeptide genes identified in the *I. scapularis* genome**.** * Predictions that need to be corrected for the reading frame. **The gene likely spans multiple scaffolds (and multiple predictions) ***Possible allelic forms of two scaffolds. NA=not automatically annotated genes. (**B**) Fourteen neuropeptide G-protein coupled receptors (GPCRs) that are expanded in number in *I. scapularis* in comparison to other arthropods. (Gulia-Nuss, 2016)

Although, the functions of most of the tick neuropeptides await experimental evidence, sequencing of the *I. scapularis* genome uncovered interesting features in tick neuropeptidergic signaling system and its potential involvement in processes relevant to their unique biology (Gulia-Nuss et al., 2016; Šimo et al., 2014). For example an interesting finding was the identification of neuropeptide genes for corazonin, eclosion hormone, cardioactive peptide and bursicon α and β (Figure 16), all known to regulate ecdysis, cuticle synthesis, hardening and tanning in insects, respectively (Nässel, 2009). The identification of specific functions of these neuropeptides in adult ticks, which do not molt, may help to uncover novel pathways for regulation of cuticle synthesis during the ingestion of large quantities of blood meal during feeding as well as other developmental processes.

Another interesting feature reported in the *Ixodes* genome was the remarkable increase (up to 10-fold) in the number of certain neuropeptide receptors (Fig. 12B), among which were insect orthologs known to regulate satiety (sulfakinin) and water balance (kinin,

tachykinin, and calcitonin-like diuretic hormone CT/DH, and corticotropin-releasing factor related diuretic hormone CRF-DH) (Nässel, 2009). Whether all of these receptors are functional genes, rather that pseudogenes, awaits experimental confirmation; however, this finding supports the hypothesis that crucial functions regulating massive body increase size and osmoregulation are accomplished via greatly increased expression of this repertoire of specific neuropeptide receptors (Gulia-Nuss et al., 2016).

3.3 POST-DOCTORAL FELLOWSHIP, (FRANCE)

3.3.1 Anti-tick vaccine targeting tick salivary gland proteins and salivary glands innervations

After seven years in the molecular entomology laboratory in KSU (USA), I enthusiastically accepted a three years postdoctoral-fellowship granted by the Laboratory of Excellence, Department of Integrative Biology of Emerging Infectious Diseases (LabEx IBEID) of Pasteur Institute, Paris, France. For this position I was selected as the best candidate among 50 applicants and joined the research team of Dr. Sarah Bonnet (INRAE) at UMR-BIPAR (INRA-ANSES-EnvA) located in Maisons-Alfort, France. This position allowed me to return to European research space and to continue in the field of tick research. Specifically, the project was focused on the development of anti-tick vaccines, leading to the disruption of the tick feeding and subsequently affecting the tick-borne pathogen transmission. For that purpose, I selected suitable candidate proteins lipocalin (IrLip1) and serine protease inhibitor (IrSPI) from the *I. ricinus* SG transcriptome database to be expressed them in insect cell line. The recombinant proteins were used to immunize the mice, and sheep models followed by the tick infestation. We predicted that immunization with both IrIrSPI and IrLip1 SG proteins will negatively affect tick feeding and/or they post-feeding development due the altered functionality for *Ixodes* SG. Although vaccination with both recombinant proteins induced a high antibodies titers in the immunized animals not effect on tick feeding disruption or development has been observed (Almazán et al., 2020a; Blisnick et al., 2017).

The discoveries in field of tick SG control I made during my PhD studies and postdoctoral position in USA (see the part 3.2) were used here as an added value for the project. Specifically, I came up with an idea to use two neuropeptides (MIP and SIFamide) involved in the control of tick SG (Fig. 4, 5) as well as tick hindgut (Fig. 9,10), as vaccine candidate (Almazán et al., 2020b). Both MIP and SIFamide mature peptides are very short in the term of their amino acid residues placing them not to be a suitable antigens for the generation of antibodies responses. Therefore, we employed the multiple antigenic peptide (MAP) strategy to in order to test signaling peptide molecules as vaccine candidates. The vaccine candidates were chemically synthetized as MAPs, in which four copies of the same peptide were formulated on a lysine-based backbone (Fig. 13A). Each branch of the MAP candidate comprised a short *I. ricinus* non-amidated neuropeptide MIP, or SIFamide, fused to an universal T-helper cell epitope, the Pan DR epitope peptide (PADRE). Both mice and sheep models were immunized with each or combination of this construct followed by the immature tick stages infestation. Although, both constructs provoked a high antibody titers in examined animal models (Fig. 13B-T) no significant effect on tick infestation, feeding, mortality have been detected. Only in the case of the immunized sheep a mild negative correlation between anti-SIFamide and MIP antibody levels and larva-to-nymph molting success for both antigens has been detected (Fig. 13H-T).

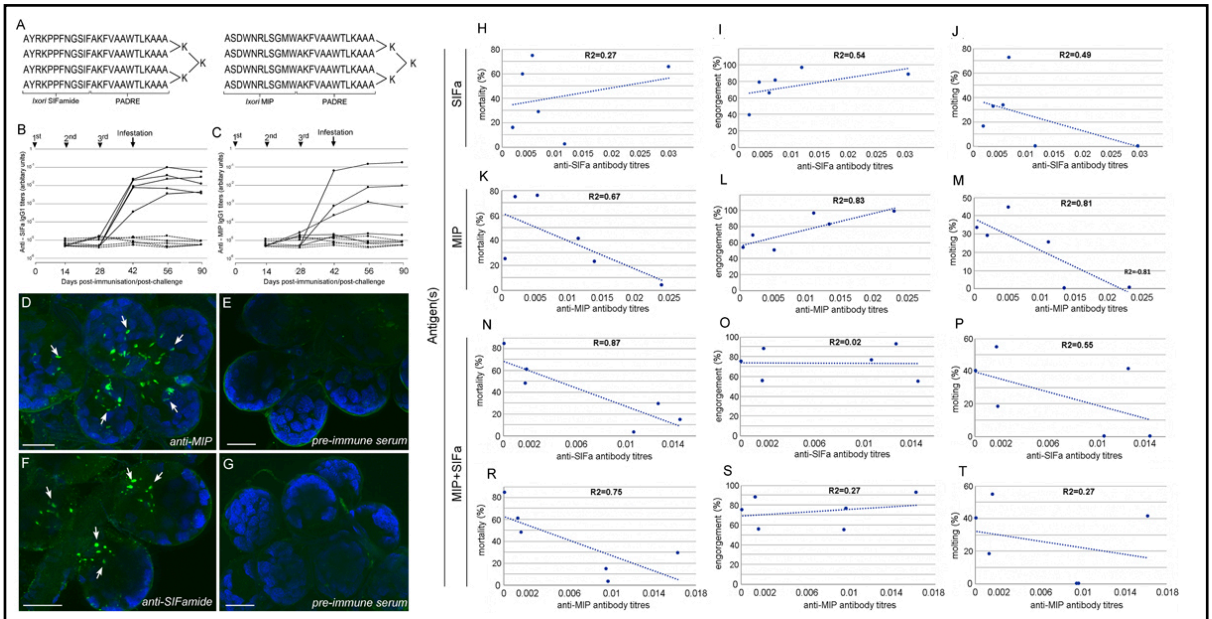
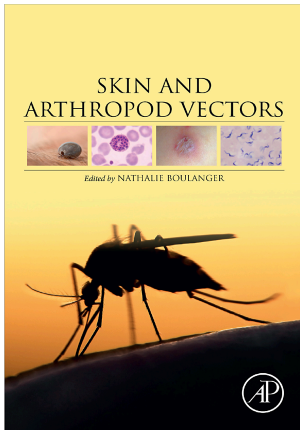


Figure 13. (**A**) Schematic depiction of multiple antigen peptides (MAPs) used in this study. Note that Ixodes ricinus mature neuropeptide B-cell epitops (SIFa or MIP) were fused to a T-helper cell epitop PADRE to construct 4-branched MAPs. (**B**) Antibody (IgG1) response to SIFamide (SIFa) and myoinhibitory peptides (**C**) in vaccinated mice. Antibody titers were determined by ELISA in serum samples collected at different time points from day 0 to day 90 against the specific peptide both in vaccinated mice (plain lines) and control mice (dashed lines) that received only adjuvant, and represented as arbitrary units (AU). Arrows indicate dates for 1st, 2nd, 3rd immunizations (days 0, 14 and 28) and tick infestations (Day 42). (**D-G**) Whole-mount immunohistochemistry on the salivary glands of an *I. ricinus* unfed female. (**D**) and (**F**) show the reaction of anti-mouse antibody generated by MIP or SIFa MAP constructs, respectively. Note that both antibodies recognized specific axon terminals (green, arrows) in acini type II and III of salivary glands. (**E**) and (**G**) are the negative controls where only pre-immune serum of mice subsequently immunized by MIP or SIFa was used, respectively. Bar is 10 µm. (**H-T**) Correlation between antibody titers against SIFa and MIP neuropeptides or mix of both (SIF and MIP) in vaccinated sheep. *I. ricinus* larvae mortality, engorgement and molting at day 45 after immunization are shown. The linear correlation coefficients (R2) and p-values are shown (N = 6). Antibody titers are represented as arbitrary units. (Almazán et al., 2020b)

Although this study did not provide the expected level of protection, it inaugurates a new strategy for protection against ticks based on the immunological targeting of key components of their nervous system. The result of this project served as a basis for obtaining a grant "*Identification of tick molecules implicated in the pathogen transmission–next generation vaccine development*" from Satt/IDF (Visions, 275 000 euros, PI: Dr. Sarah Bonnet). As a part of this project an extensive review on the role of tick SG in pathogen transmission in *Frontiers in Cellular and Infection Microbiology* having up to today more then 140 citations has been published (Šimo et al., 2017). In addition I also contributed to the book chapter *Tick Saliva and Its Role in Pathogen Transmission* in the book *Skin and Arthropod Vectors* (Bonnet et al., 2018) (see on right site of this paragraph)



Although my postdoctoral fellowship in was scheduled for 3 years, after one year and half, I effectively vacated this position due to the possibility to apply for the direct INRAE CR1 open competition. A successful defense of my project, focusing on molecular tick neurophysiology, in front of broad committee

composed of a INRAE and external scientists in Paris gave me an opportunity to drive my own experimental process as an independent researcher in my current research unit UMR-BIPAR (Maisons-Alfort).

3.4 PRINCIPAL INVESTIGATOR (INRAE, FRANCE)

My ultimate goal as an INRAE CRCN researcher (previously CR1) was to build and internationally recognized laboratory focusing on the physiological mechanisms underlying the pathogen transmission by tick vectors as this topic was presented during my INRAE CR1 interview process. Currently, none tick research program on tick molecular physiology or their signal transduction system exists, neither in France, nor in the rest of Europe. For this reason, there is a strong potential that my research program merging the topic of tick physiology with tick-borne pathogen is greatly enriching tick research at both national and international levels.

During the first year in INRAE, I have made an intensive effort to firmly establish the essential tools, including a vast majority of the techniques for investigation of tick molecular physiology in the laboratory. With the great help of the two funding resources I obtained, ENVA research grant (8529 euro) and INRA young scientist grant (10 000 euro), I established various tools directly relevant to my research topic and simultaneously enriched the spectra of the techniques in UMR-BIPAR. This includes: molecular cloning, immunohistochemistry, *in situ* hybridization, RNAi techniques, qRT-PCR, imaging, heterologous expression of GPCRs, novel system of tick rearing and feeding, and tick nano-injection. Here I would like to highlight the animal-friendly novel technique of tick feeding (Almazán et al., 2018, Mateos-Hernandéz et al., 2020a) I established in the institute, which has been immediately adapted by multiple tick researchers across the different research groups the UMR-BIPAR unit as well as other institutions in France. Technical independence was a crucial step in order to effectively achieve the vast majority of my scientific goals and simultaneously build in UMR-BIPAR the core French/European laboratory focusing on tick signaling systems and their relation to tick borne-pathogens.

My current activities as CRCN INRAE researcher include primarily the basic research focusing on the roles of signaling pathways in tick physiology. The research I am conducting is highly experimental and includes numerous modern techniques directly relevant to examined topics. The major goal of my research is to improve the molecular understanding of the host – vector interactions and elucidate how tick neuropeptides, protein hormones, neurotransmitters and their receptors may be manipulated to control of these important vectors of pathogens. Recently I have stepped into the field of tick borne pathogens and together with Dr. Alejandro Cabezas-Cruz (INRAE, UMR-BIPAR) merged tick neurophysiology area with tick signaling systems perfectly fitting to the One-Health concept for optimization of the health of humans, animal and environment. This merging process led to the creation of "NeuroPaTick" (Neuro – Neurophysiology, Pa – Pathogens, Tick – Tick, www.neuropatick.com) as a new area in tick research, established for the first time in the UMR-BIPAR. Such a direction has opened a new area of research field having the potential to uncover vulnerable spots in tick physiology with the strong potential to lead to the development of better control measures of these medically important arthropods and the pathogen they transmit. The main current project in my laboratory are detailed below.

3.4.1 Ultrastructural mapping of the innervation in the tick *Ixodes ricinus*

The main goal of this project was the ultrastructural examination of my previously identified specific axonal projections expressing neuropeptides or neurotransmitters receptors (Fig. 14) within the SG of the tick *I. ricinus.* The current ultrastructural studies of tick SG focus primarily on the microscopical detail of particular cells in the acini (Coons et al., 1994; Fawcett et al., 1981; Needham et al., 1990) however none of them used specific molecule labeling to visualize them in this tissue. The structure of the tick SG is very complex and distinguishing between different cell types in particular type of alveoli using classical immunohistochemistry mostly relies on nuclei visualization with DAPI staining (Fig.3B-I, 4D-I, 5D-H, 8A-L). An employment of immuno-transmission electron microscopy (immuno-TEM) approach was therefore critical in order to derive functional implications based on the association of particular molecules with particular cell type(s). I coordinated this project in collaboration with Dr. Marie Vancova from the electron microscopy laboratory in Biology Centre of Czech Academy of Science in Czech Republic and the results led to the first description of the SG ultrastructure of the European vector *I. ricinus* and highlighted the association of the axonal projections with different highly convoluted SG cells. Specifically the ultrastructural investigations of associations of SIFamide neuropeptide, SIFa_R, neuropeptide PDF, and the InvD1L, with acinar cells of *I. scapularis* female was performed. The current study reveals several novel ultrastructural architectural features of SG type II and III acini from unfed *I. ricinus* females (Vancová et al., 2019).

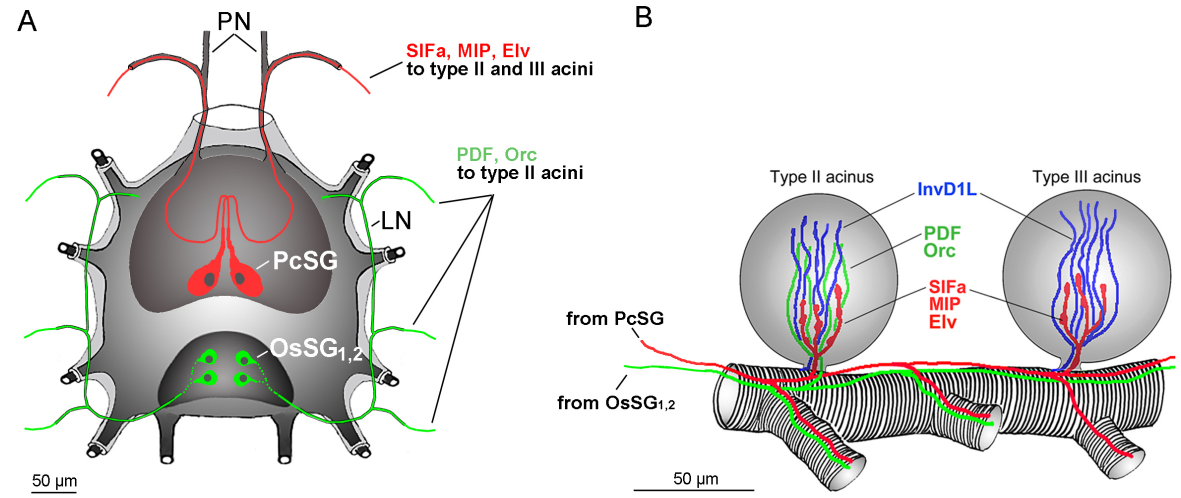


Figure 14. Neuronal projection innervating the salivary gland (SG) of *I. scapularis* detected up to today. (**A**) Tick synganglion with peptidergic neurons identified as sources of SG innervation. Giant protocerebral neurons PcSG (red) expressing three different neuropeptides SIFa (SIFamide), MIP (myoinhibitory peptide) and Elv (elevenin) innervate basal parts of types II and III acini shown in (**B**). Another set of opisthosomal neurons $OsSG_{1,2}$ (green), reacting with antibodies against PDF (pigment dispersing factor) and Orc (orcokinin) innervate exclusively type II acini shown in (**B**). Abundant axonal projections expressing InvD1L (invertebrate-specific D1-like dopamine receptor) and innervating (blue in **B**) both type II and III acini have an unknown origin. Note that image B shows only one single type II and type III acinus attached to the salivary duct. Axons running along the main duct and different axon terminals entering the acini are shown in different colors. PaN – palpal nerve, LN – lateral nerve. A is from (Sonenshine and Šimo 2021). B is modified from (Vancová et al., 2019)

Because the written text of the ultrastructural results may cause a difficulties for non-specialist readers to understand the described features, I provide a simplified bullet-points summary of the results linked to the multiple TEM images and final schema (Fig. 17) for easier understanding:

- Basal epithelia cells (ECs) closely surround the acinar duct and its valve (Fig. 15, 17). A single mesh-like myoepithelial cell (MC) lines the entire acinar lumen and extends its finger-like projections between granular cells toward the basement membrane (Fig.15A-D, 17). Interstitial extensions of abluminal epithelial cells (AEC) radiate between the granular cells toward the acinar lumen to make contact with the MC and/or ECs (Fig. 15A-O, 17). AECs are never in direct contact with the acinar lumen (Fig. 15A, 17). The MC is in contact with all acinar cells (Fig. 15A, 17).
- The acinar valve is covered by two layers of cells: apical EC extensions line the entire length of the valvular arms, while the MC cell overlays nearly half of these extensions (Fig. 15A,B, 17). Both the MC and ECs possess abundant microtubules, primarily located near the acinar valve (Fig. 15B).
- SIFamide (SIFa) and SIFa_R are associated with both ECs and the MC (Fig. 16, 17). Large SIFa-axons are encapsulated by EC plasma membranes around the acinar duct or the MC cell adjoining the region above the acinar valve (Fig. 16A-M). Accordingly, SIFa_R reactions were also detected, always in close proximity to SIFa-axons (Fig. 16 D-M, 17). Specifically, SIFa_R is expressed on EC canaliculi and the MC plasma membrane, both near the apex of the acinar duct and its valve (Fig. 16 D-M, 17).
- InvD1L-axons run the entire length of the acinus, in close proximity to its lumen (Fig. 16N-Z). These axons are primarily enclosed by the ablumenal membrane of MC and terminal AEC extensions . These axons are occasionally in contact with apical granular cells.
- PDF-axons surround the lumen of type II acini only. These axons reach the apical region of the acini, and are in direct contact with the MC plasma membrane and terminal AEC extensions (Fig. 17).
- In type III acini, the axon expressing unknown substance(s) and containing electron-lucent vesicles runs along the SIFa- and InvD1L-axons, and is likely only specific to this type of acini (Fig. 16U-Z).

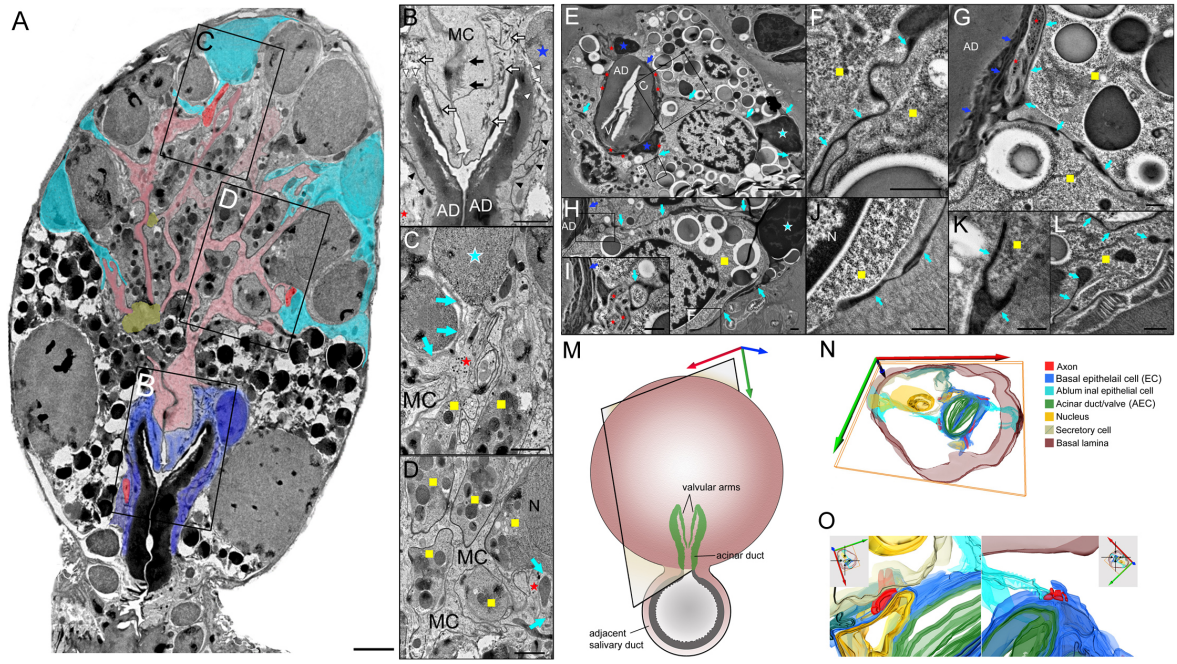


Figure 15. Transmission electron microscopy image of type III (**A-D**) and type II (**E-O**) acinus from unfed *I. ricinus* female salivary glands highlighting the cellular composition and associated axons. (**A**) Cross-section through the middle of the acinus reveals common axon positions (red), basal epithelial cells (ECs, dark blue), myoepithelial cell (MC, pink), ablumenal epithelial cells (AECs, aqua-blue) and lumen (yellow). Different granular cells at both basal and apical regions

Figure 15. ***continued:*** of the acinus are left in black and white. Insets in A are magnified in (**B,C,D**). (**B**) The acinar valve region. Apparent microtubules (black arrows) in the MC and (white empty arrows) in ECs are shown. EC labyrinthine junctions (black arrowheads) are closely associated with the acinar duct (AD). Basal canaliculi (white arrowheads) of ECs are evident on the AD apex. The blue asterisk shows the nucleus of the EC. The basal axon is indicated by the red asterisk. (**C,D**) The axons (red asterisks) are in direct contact with the MC's finger-like projections which radiate between granular cells (yellow squares) and fine projections of AECs (aqua-blue arrows in H,I). Aqua-blue asterisk in C shows the nucleus of AEC. (**E-G**) Multiple axonal projections (red asterisks) run closely along the acinar duct (AD) and are enclosed by basal epithelial cells (ECs, blue arrows) and the extensions of AEC (aqua-blue arrows). Nuclei of ECs (blue asterisks in E) and of AEC (aqua blue asterisk in A). Insets in E are magnified in (**F,G**). AEC extension-aqua-blue arrows, granular cells-yellow squares. (G) Detail of axons surrounded by the ECs (blue arrows) and AEC projection (aqua-blue arrows). (**H-L**) Different depth section of the region shown in (E). The interstitial projections (aqua-blue arrows) of AEC, located under the basal lamina (nucleus of AEC is labeled with aqua-blue asterisk), reach the region of the acinar duct, and along with ECs (blue arrows) enclose the axons (red asterisks). Inset in (D) is magnified in (F). (F–H) Under the acinar basement membrane, different AEC projections (aqua-blue arrows) invaginate granular cells (yellow squares). (**M-O**) Schematic 3D model of basal region of the type II acinus, reconstructed from serial ultrathin sections. (M) Schematic image of acinus indicating the position of the reconstructed area highlighted in panel (**N**). (B) Axonal projections (red) running along the acinar duct (green) are encapsulated by ECs (blue) and projections of AECs (aqua-blue). (**O**) Magnified regions of image (N), with coordinates shown in their insets. Note that ECs separate the axons from direct contact with the acinar duct. Scale bars: 5 µm (A,E), 2µm ((B-D) 500 µm (F-I) 200 nm (J-L). (Vancová et al., 2019)

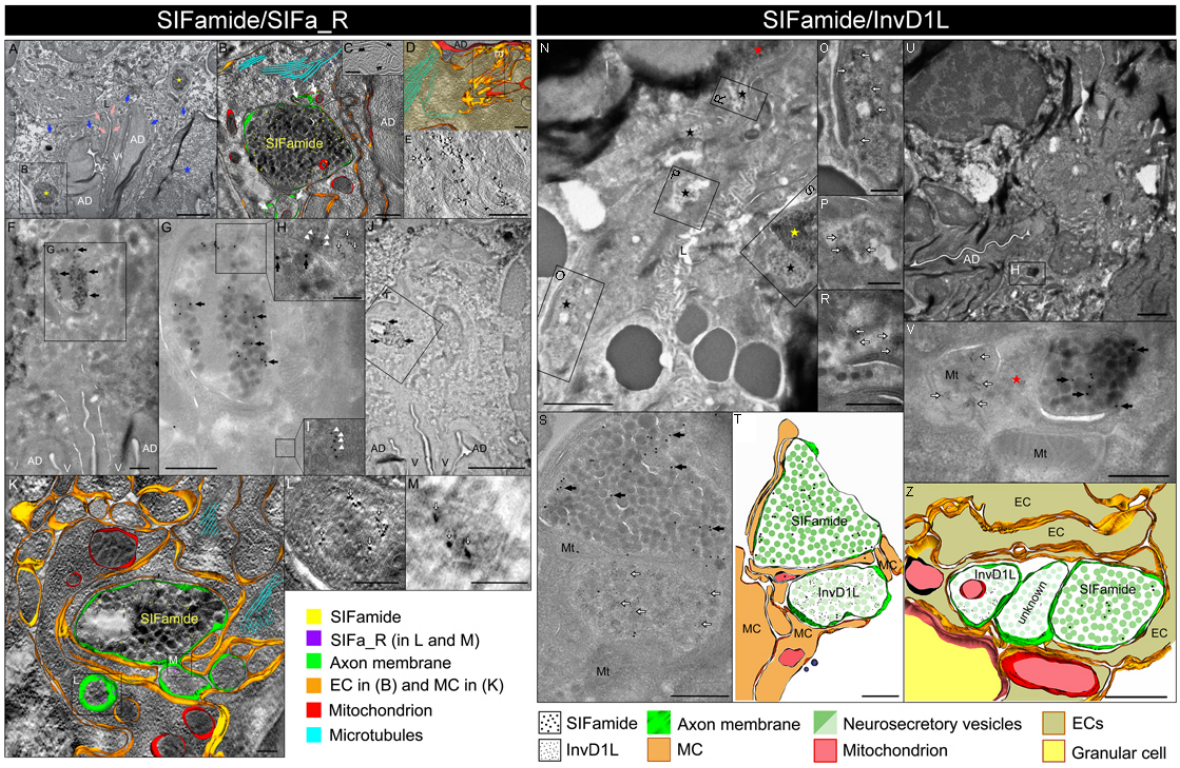


Figure 16. Left panel: Transmission electron microscopy (TEM) image showing immunogold labeling of SIFamide and SIFamide receptor (SIFa_R) in type III acinus of unfed *I. ricinus* female salivary glands. (**A**) The SIFa-axons (yellow asterisks) were found in close proximity to the acinar duct (AD) and its valve (V). Myoepithelial cell (MC, pink arrows) and basal epithelial cells (ECs, blue arrows) surround the acinar valve. The inset in A is magnified in (B). (**B**) Basal SIFa-axon containing electron-dense neurosecretory vesicles (yellow dots indicate the reaction with SIFamide antibody). Four small axons (white arrows) containing electron-lucent vesicles are associated with the large SIFa-axon. Note that the axons (green) are separated from the acinar duct (AD) and are enclosed by highly convoluted ECs (gold) forming septate junctions (black arrowheads in **C**). (**D,E**) SIFa_R staining (white empty arrows in E) is observed on the EC canaliculi in the AD apex region. Black arrowheads in E show EC membranes. (**F–M**) Double labeling of SIFamide (black arrows) and SIFa_R (white empty) in the region above the acinar valve. Insets in F and J are magnified in (**G**) and (**K**) respectively. Insets in K are magnified in (**L,M**). Note that two different SIFa_R reactions were recognized in close proximity to the prominent SIFa-axon (**H,I,K–M**). First, SIFa_R-positive vesicle-like structures (white arrowheads in H,I) and the MC plasma membrane (white arrowheads in H). Second, the small axons containing electron-lucent vesicles accompanying the SIFa-axon were also positive for SIFa_R (white empty arrowheads in H,L,M). Scale bars are 2 µm (A,J), 500 nm (B,F,G), 200 nm (D,E,H,K,L), 100 nm (C,M). **Right panel:** TEM image showing double immunogold labeling of

Figure 16. *continued:* SIFamide and invertebrate-specific D1-like dopamine receptor (InvD1L) in both type II (N–T) and III (O–Z) acini of unfed *I. ricinus* female salivary glands. (**N**) Cross section of type II acinus highlighting the axons running close to the acinar lumen (L). Five InvD1L-axons (black asterisk) containing electron-lucent vesicles, one SIFa-axon (yellow asterisk) containing electron-dense vesicles and one unlabeled axon (red asterisk) containing electrondense vesicles were recognized. Insets in (N) are magnified in (**O-S**). InvD1L- (white empty arrows) in (**O–S**) and SIFamide- (black arrows) in (**S**) reactions are shown. (**T**) 3D model of (S) emphasizing the axons and surrounding MC. (**U**) Basal region of type III acinus highlighting three different axons running alongside close to the acinar duct (AD). Inset in (U) is magnified in (V). InvD1L-axons (white empty arrows) containing electron-lucent vesicles, SIFa-axon (black arrows) containing electron-dense vesicles and an unlabeled axon (red asterisk) containing electron-lucent vesicles were detected. (**Z**) 3D model of (V) emphasizing the axons and surrounding ECs. Scale bars: 2 µm (A,G), 500 nm (B–F,H,I). (Vancová et al., 2019)

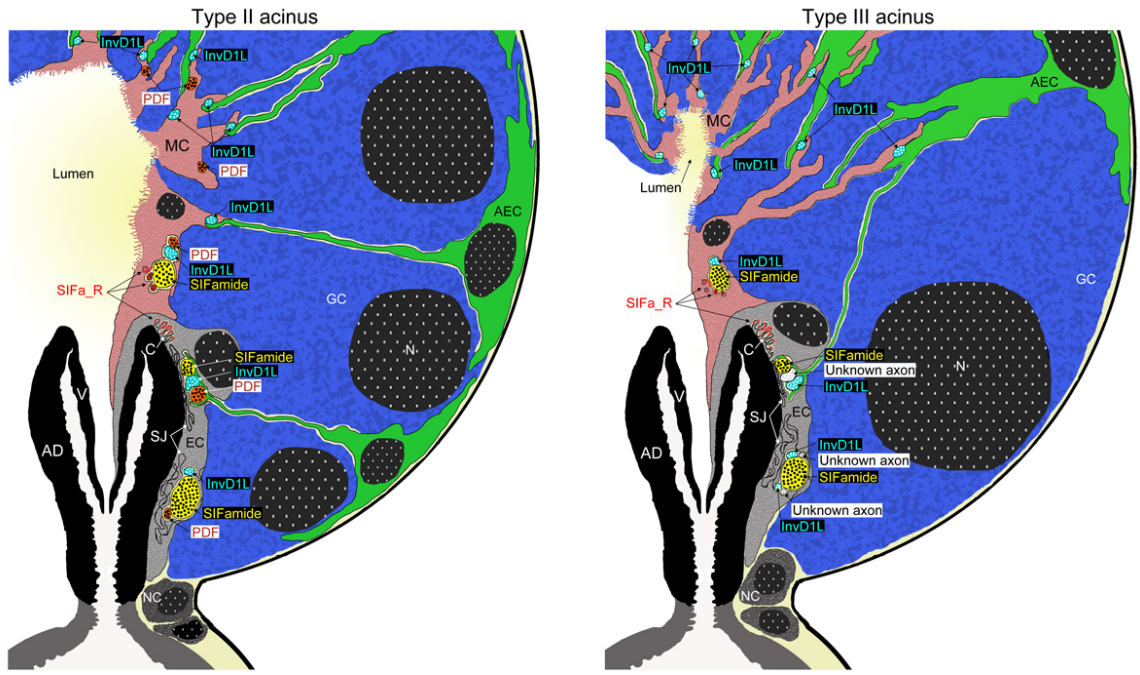


Figure 17. Schematic illustration demonstrating cross sections of type II and III acini from unfed *I. ricinus* female salivary glands highlighting the organization of acinar cells and their putative associations with different axonal projections. SIFamide-positive axons (yellow), pigment dispersing factor-positive axons (PDF, brown/ orange), invertebrate-specific D1-like dopamine receptor-positive axons (InvD1L, aqua-blue). SIFamide receptor (SIFa_R, red): on plasma membrane and vesicle-like structure (upper arrow) of the myoepithelial cell (MC, pink), axons accompanying the SIFa-axon surrounded by the MC (middle arrow) and canaliculi (lower arrow) of basal epithelial cell (EC, grey). Note that acinar valve (V) is covered by EC extensions and overlaid by the MC. AEC, ablumenal epithelial cell: green; GC, granular cell: blue; NC, neck cells: dark grey; N, nucleus: dotted black; AD: acinar duct; C: canaliculi of ECs; SJ: septate junctions. (Vancová et al., 2019)

Our results indicate that a rich network of various axon types, differing in their lengths, thicknesses, and expressed substances, form intimate contacts with multiple different acinar cell types, highlighting the complexity of the neural regulatory mechanisms in hard tick SGs. The outcome has been published in the *Nature* publisher group journal *Scientific Reports* (Vancová et al., 2019). Besides this publication, we successfully collaborate with Electron microscopy laboratory of Biology Centre in Czech Republic for other several projects related to the ultrastructure of tick SG and feeding apparatus-related structures (Kim et al., 2017, Vancová et al., 2020). In addition, I took the opportunity to make a connection between my European research network and the former KSU (USA) laboratory where I served as a key interface and coordinator for multiple projects related to tick signaling systems.

3.4.2 Cholinergic signaling in tick salivary glands

Despite my previous discoveries in the field of tick SG control the crucial regulatory factors and their precise physiological mechanisms are still poorly understood. The aim of this project is to explore for the first time the molecular nature of cholinergic signaling in SG of the tick *I. ricinus* and *I. scapularis* ticks, both principal vector of *Borrelia burgdorferi* causing Lyme borreliosis in Europe, and North America respectively (Rizzoli et al., 2014; Walker, 1998). Obtained preliminary data created a baseline for obtaining two young scientist grant (INRAE and ENVA, PI Ladislav Šimo, together total 18.529 euros). In addition in 2017 our preliminary results served as a baseline for the young researcher ANR (Acronym: CholiTick) preproposal followed by the full proposal submission. The full proposal was very highly ranked and was recommended for funding by the ANR panel, but unfortunately it was transferred to the supplementary list (*Liste complémentaire*). Furthermore, the topic also allocated one postdoctoral researcher and one master 2 student (Baptiste Defaye, University of Limoges).

The initial inspiration to develop this study was the surprisingly poor understanding of how cholinomimetic agent pilocarpine-mediated SG fluid secretion occurs, especially considering this is such a common SG fluid secretion induction process in many tick species worldwide(Kaufman, 1976; Kaufman and Harris, 1983; Patton et al., 2012). Although acetylcholine (ACh) is known to be a predominant neurotransmitter in insects (Florey, 1973; Pitman, 1971), the presence of a cholinergic system in ticks has only been chemically validated (Baxter and Barker, 1998), thus the effects of cholinomimetic ligands at specific tick body sites remains an unknown black box. In addition, the molecular characterization of choline acetyltransferase (ChAT), the enzyme involved in ACh synthesis, and the vesicular acetylcholine transporter (VAChT), responsible for loading ACh into secretory granules in pre-synaptic cells (Erikson et al., 1994) remain understudied. ACh acts via two types of receptors: ionotropic nicotinic acetylcholine receptor (nACh_R) and metabotropic mACh_R (Sattelle, 1980). To date, only the nACh_R alpha subunit of the cattle tick *Boophilus microplus* has been identified (Lees et al. 2014), and there is only indirect evidence for mACh_R in ticks (Kaufman, 1978; Patton et al., 2012; Sauer et al., 1995). Specifically, when the mACh_R agonist pilocarpine is injected *in vivo* in the presence of the synganglion, it induces robust long-lasting SG secretion, whereas it fails to induce saliva from isolated SGs in the absence of the synganglion (Kaufman, 1978; Kaufman and Harris, 1983).

In our project we demonstrated that cholinergic signaling between synganglion and SG is very complex, and may hold vital clues to understanding key aspects of SG functionality (Mateos-Hernandéz et al., 2020b). For the first time we reconstructed the organization of *Ixodes* cholinergic gene locus (Fig.18. A-C) consisting of two alternatively-spliced transcripts encoding ChAT and VAChT, in a configuration similar to that identified in other metazoans (Candiani et al., 2008). Using antibody against ChAT we identified distribution of cholinergic neurons in synganglion along their axonal projections reaching exclusively acini type I in SG (Fig. 18 E-T). Two opistosomal (posterior lobe in synganglion) neuronal cells (OsSG) are recognized as a source of this innervation (Fig. 18 E-T). The *in situ* hybridization confirm the expression of mRNA encoding choline acetyltransferase (ChAT) and vesicular acetylcholine transporter (VAChT) in OsSG neurons (Fig. 18F,G). ChAT-positive axonal projections (from OsSG neurons) exit the synganglion through the opisthosomal nerve (Fig. 18, E, H-J, T), travel along the salivary duct surface, and terminate at type I salivary acini (Figs. 18 K-T). ChAT-positive axons enter each single acinus through the neck region and then branch into multiple axon terminals containing varicosities (Fig. 18 N-S). Based on these findings we predicted that

acini type I in *Ixodes* SG are the target for cholinergic axonal signaling and thus should express cholinergic receptors.

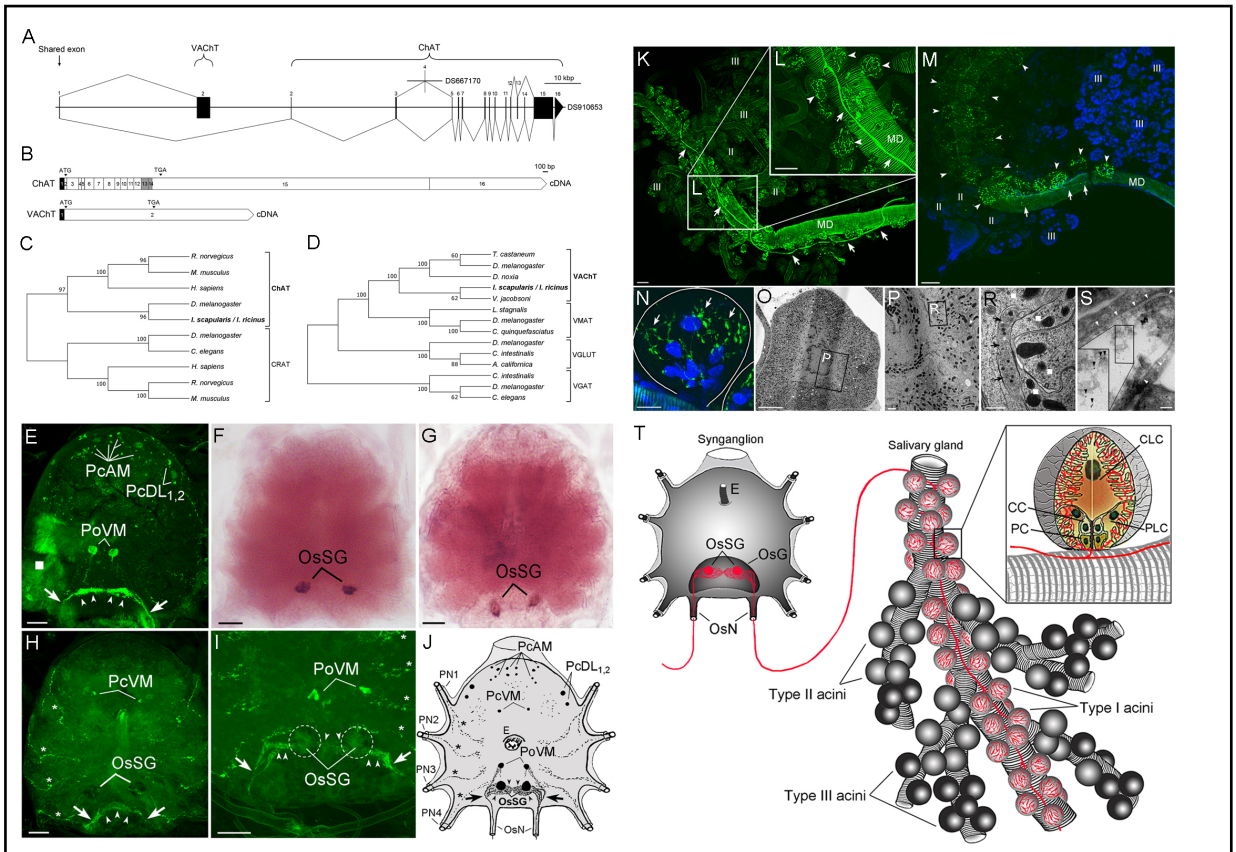


Figure 18. (**A**) Structure of the *I. scapularis* cholinergic gene locus. Horizontal lines represent introns, while vertical lines and black boxes represent exons (numbered). Splicing between exons is indicated with diagonal lines. Exon 1 is shared by both ChAT and VAChT. The second exon of VAChT (that spans its entire ORF) lies within the first intron of ChAT. Exons numbered 13 and 14 are the two mutually exclusive exons. (**B**) cDNA schematics of ChAT and VAChT indicating the proportional lengths of each exon (numbers). The black color indicates the region encoded by the shared exon. The grey color in ChAT indicate the two possible ChAT transcript variants (A and B) based on the mutually exclusive exons shown in (A). (**C,D**) Phylogenetic relationships of ChAT (**C**) and VAChT (**D**) of *I. ricinus*/*I. scapularis*. CRAT carnitine acetyltransferase, VMAT vesicular monoamine transporter, VGLUT vesicular glutamate transporter, VGAT vesicular GABA transporter. Whole-mounted immunohistochemistry (IHC) of ChAT (**E,H,I**) and whole-mounted *in situ* hybridization (ISH) of *chat* and *vacht* (**F,G**) in synganglia of unfed *I. ricinus* females. (**E**) dorsal view on synganglion, *chat* (**F**) and vacht (**G**) of ISH stained ventral view of synganglion. (**H**) Ventral side of the I. ricinus synganglion after ISH of *chat* followed by IHC on the same specimen. Note that the dark spots are the SGs innervating opistosomal neurons (OsSG) visualized in ISH as seen in the confocal image. (**I**) Detail of the ventral posterior part of the synganglion highlighting the OsSG neurons (in dotted circles) and associated axons. (**J**) Schematic illustration of the *Ixodes* synganglion highlighting the observed cholinergic neurons and their axons. Arrowheads—axonal clusters likely belonging to the OsSG neurons; arrows—axons exiting the OsSG cells and entering the opistosomal nerves (OsN), asterisks— lateral pedal axons, PN1-4—pedal nerves 1–4, E—esophagus; white square in (E) shows the disrupted part of the synganglion that facilitated the diffusion of the antibody inside the tissue. Scale bars are 50 µm. Immunostaining of ChAT in *Ixodes* salivary gland (SG). IHC of *I. ricinus* (**K,L**) and *I. scapularis* (**M**) unfed female SG. Note that cholinergic axons (green) run along the main salivary duct (arrows) and terminate exclusively within the acini type I (arrowheads). Roman numbers II and III indicate type II and III acini located more distally from the main salivary duct (MD). (**N**) Detailed view of single type I acinus, highlighting its arborized cholinergic axon terminals (green, arrows). (**O**) Transmission electron microscopy (TEM) image showing the acinus I. Insects in O and **P** are magnified in P and Rm respectively. (**R**) Axons (arrows) containing electron-dense vesicles. (**S**) TEM image showing immunogold ChAT antibody labeling (black arrowheads) of the axon within the type I acinus in SG. White arrowheads show the axolemma. Blue in M and N is DAPI staining for nuclei. Scale bars are 50 µm in (K–M), 10 µm in (N, O), 1 µm in (P), 500 nm in (R), and 100 nm in (S). (**T**) Schematic illustration of the cholinergic connection between the Ixodes synganglion and salivary gland. The cholinergic axons (red) originating from OsSG neurons exit the synganglion via the opistosomal nerves (OsN) to reach the type I acini exclusively. (B) Schematic detailing the distribution of cholinergic axons (red) within the type I acinus. Peripheral lamellate cells (PLC) and the central lamellate cell (CLC). CC circumlumenal cell, PC peritubular cell, E esophagus, OsG opistosomal ganglion. (Mateos-Hernandéz et al., 2020b)

Indeed two different types of mAChRs (type A and B) were identified in *I. scapularis* genome (Fig. 19 A) and found to be expressed in SG. Both of these receptors we functionally tested in our heterologous expression systems. The mAChR-A was highly sensitive to different doses of ACh and muscarine while less to the tick non-endogenous drug pilocarpine (Fig. 19B-E). The mAChR has been suggested to play a crucial role in tick SG physiology since the cholinomimetic agent, pilocarpine, was the first pharmacological compound found to stimulate tick SG secretion in vivo (Howell C.J., 1966; Tatchell, 1967). The hypothesis that mAChR may be a cholinoceptive site for salivation has also been confirmed by effectively blocking pilocarpine-mediated fluid secretion with a typical mAChR-A antagonist (Kaufman, 1978) atropine, that was also confirmed in our pharmacological experiment of mAChR-A (Fig. 19 F,G). Interestingly, we showed that sensitivity of mAChR to pilocarpine agonist, had approximately 25 x lower activity for this drug compared to ACh, indicating that pilocarpine is a non-potent activator of the receptor. Despite the enormous effort in our laboratory we failed to activate mAChR-B likely due the downstream receptor incompatibility in our expression system. Thus, taken together, more thorough investigations are required to conclude whether pilocarpine-mediated SG fluid secretion is regulated via mAChR(s) or other system(s).

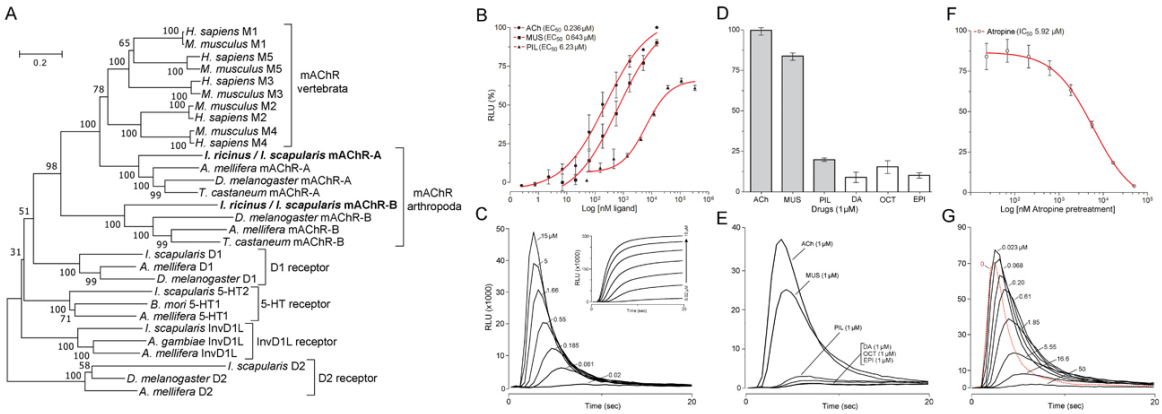


Figure 19. (A) Phylogenetic relationship between vertebrate and arthropod mAChRs including mAChR-A and mAChR-B from *I. scapularis/I. ricinus*. D1-D1 dopamine receptor, InvD1L-invertebrate-specific D1-like dopamine receptor, 5-HT-receptor 5 hydroxytryptamine (serotonin) GPCR, D2-D2 dopamine receptor. Bioluminescent aequorin reporter assays for mAChR-A expressed in CHO-K1 cells alongside the human Gα15(16) subunit. (**B**) Dose–response curves to various doses of acetylcholine (ACh), muscarine (MUS), and pilocarpine (PIL). (**C**) Representative 20-s typical luminescent responses to different doses of ligand (muscarine in this case). Inset in (C) shows the integrated relative luminescent values calculated from 40 intervals within the 20 s responses. (**D**) Luminescent responses to different drugs at 1 µM concentration. Dopamine (DA), octopamine (OCT), and epinephrine (EPI). (**E**) 20-s luminescent responses to 1 µM drugs. (**F**) Typical dose–response curve of antagonistic atropine activity. (**G**) Representative luminescent responses to 10 µM ACh after pre-incubation with different atropine concentrations. The bars in (B,D,F) indicate the standard error for three replicates. (Mateos-Hernandéz et al., 2020b)

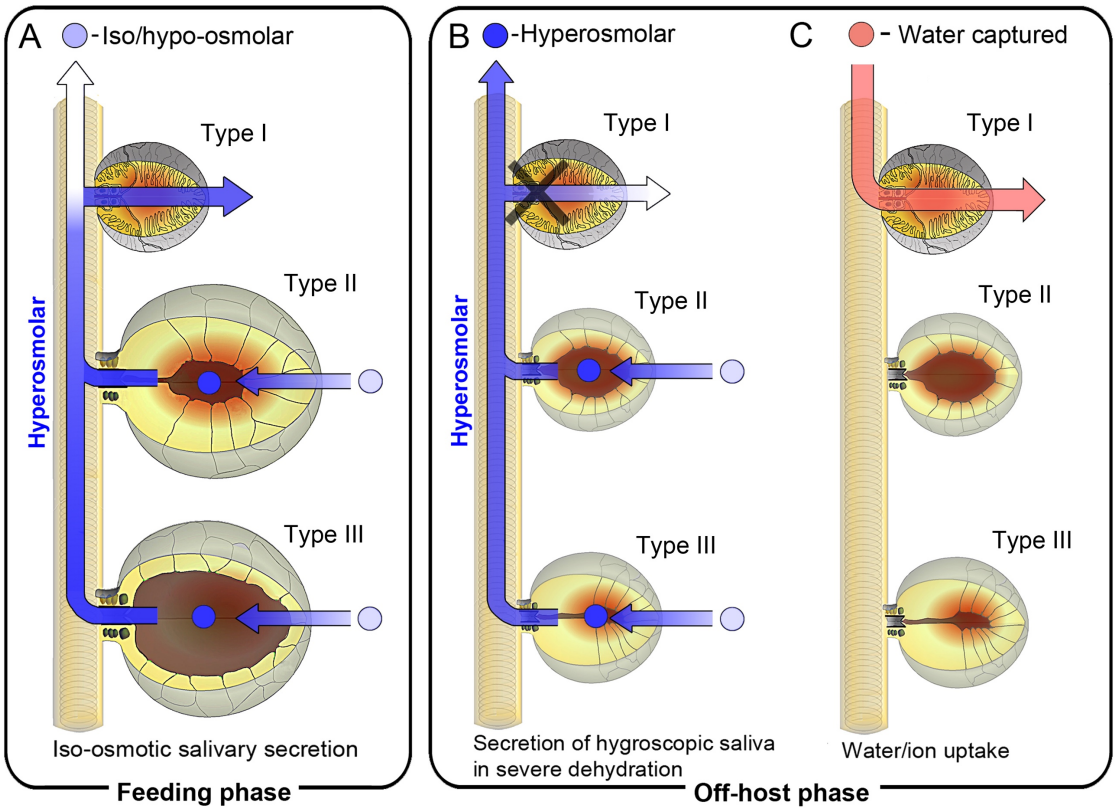


Figure 20. A model explaining roles of specific acini types in *Ixodes scapularis*. (**A**) The process for iso/hypo osmolar salivary excretion in feeding ticks. (**B**) Production of hyperosmolar saliva for capturing water molecules. (**C**) Uptake of water captured in the hygroscopic salivary secretion. (Kim et al., 2019)

The specific activities of individual acini of tick SG mediate diverse functions that ensure the tick's biological success during both on- and off-host periods (Bowman et al., 2008; Kim et al., 2019). Studies examining the physiology of tick SG have focused primarily on the on-host tick feeding period, during which the secretory activities of the types II and III acini are most apparent (Binnington, 1978; Kim et al., 2019; Meredith and Kaufman, 1973). Based on the investigation of tick SG function by our group, we suggested that when feeding, these structures produce hyperosmolar primary saliva that passes via ducts to the anterior part of the gland where type I acini absorb ions resulting in iso-osmolar saliva (Fig. 20A). During the fasting period, when the tick undergoes severe stress due to desiccation, the absorptive function of type I acini is shut down and hygroscopic hyperosmolar saliva is secreted (Fig. 20B,C) onto the hypostome surface to from a crystalized matrix. In a more favorable environment, this deposit is deliquesced by atmospheric water, and the type I acini absorb water from this diluted saliva (Fig. 20B), thereby keeping the ticks rehydrated (Kim et al., 2014, 2017, 2019). As mentioned above we showed that cholinergic axon terminals reach the lamellate cells of *Ixodes* acini type I (Fig. 18K-T) and we predicted that stimulation of putative postsynaptic mAChR, triggers the resorptive activities of these structures.

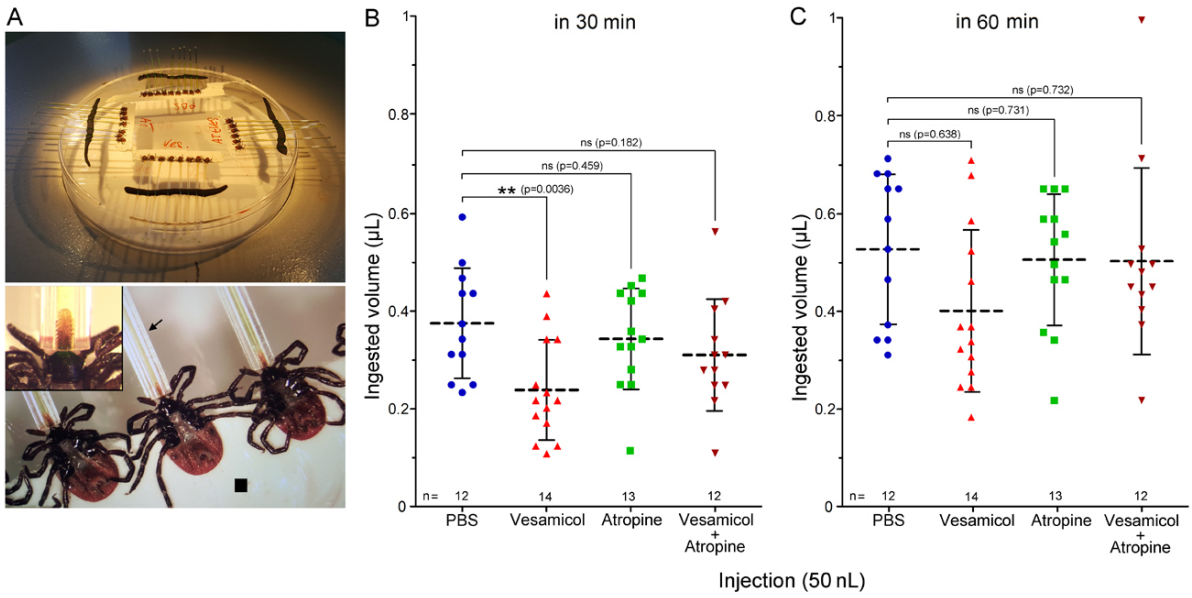


Figure 21. Effects of vesamicol and atropine on volume of water ingested by desiccated *I. ricinus* females. (**A**) Experimental set-up of forced water ingestion by desiccated ticks. Four different groups of *Ixodes* females (upper panel) were connected to glass microcapillary tubes (arrow, total volume 1 µl) via their hypostomes (lower panel). The black square shows the double-sided adhesive tape to which the ticks were attached upside-down. Inset in lower panel shows detail of the hypostome inserted into the glass microcapillary. (**B**) Ingested water volume by ticks pre-injected with PBS or different drugs at 30 min. (**C**) Ingested water volume by the same group of ticks as in (**B**) at 60 min. Each symbol in (**B,C**) indicates an individual Ixodes female. The horizontal dotted line represents the mean, and the bars the standard deviation. The experiment represents two biological replicates. (Mateos-Hernandéz et al., 2020b)

Therefore, we designed an experiment to test whether disrupting synaptic ACh release and/or blocking postsynaptic mAChR in type I acini may affect water ingestion in severely dehydrated ticks (Fig. 21). Although we observed substantial variation in the amount of ingested water between individual *Ixodes* females, significantly less volume was ingested by ticks treated with vesamicol (Fig. 20), a drug inhibiting ACh uptake by synaptic vesicles and thus reducing its release into the synapse (Salin-Pascual and Jimenez-Anguiano, 1995). Interestingly, ticks treated with the mAChR-A antagonist, atropine, ingested approximately the same volume of water as control ticks. Similarly, no effect on ingestion volume was observed in ticks treated with the vesamicol/atropine mixture (Fig 21).

The outcome has been published in the *Nature* publisher group journal *Scientific Reports* (Mateos-Hernandéz et al., 2020b) and both, the postdoc (Lourdes Mateos-Hernandéz) and Master 2 student (Baptiste Defaye, University of Limoge), I supervised, shared the first co-authorship. The project represents a very successful collaboration between the UMR-BIPAR, UMR-Virology, Kansas State University (USA) and Biology Centre of Czech Academy of Sciences (Ceske Budejovice Czech Republic). Our study is the first report describing cholinergic innervation of tick SG and suggests its role in water absorption by desiccated ticks. This novel system we uncovered may lead to the development of environmentally benign strategies to control multiple tick species around the globe. For example, due to the moisture absorbing capabilities of acini type I, described by our group, may lead to a design of naturally delivered acaricides directly targeting the mAChRs in tick SG having a fatal effect on ticks during off- stages.

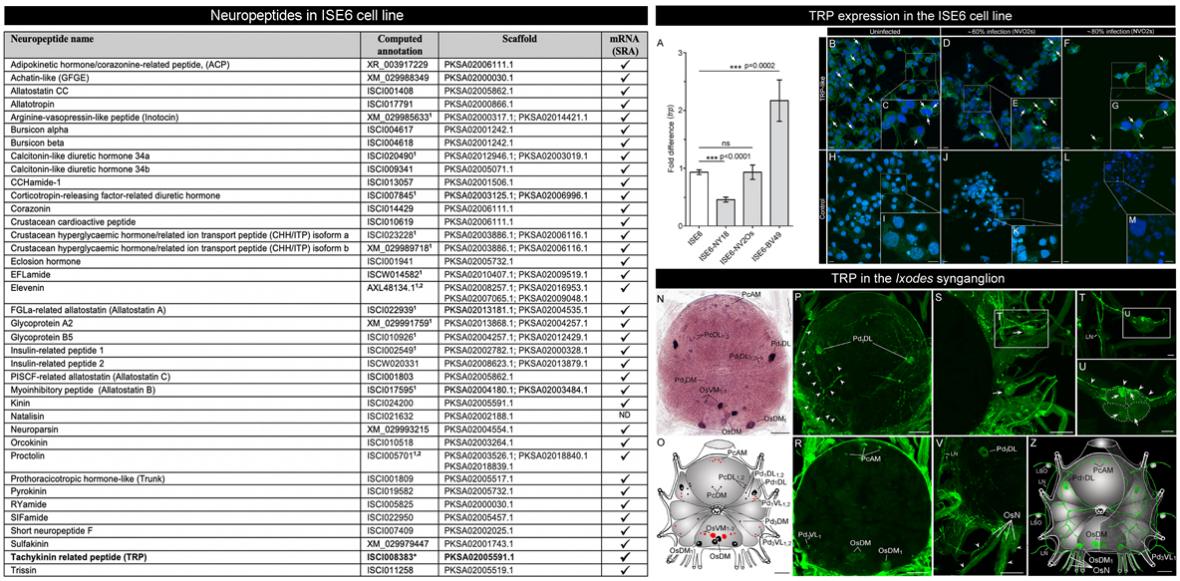
3.4.3 Neural basis of tick pathogen interactions

The critical point in my career, since hired by INRA, has been inclusion of tick-borne pathogens into my purely tick-physiology oriented research. This inclusion, was highly successful and led to the development and establishment of a completely new area in tick research in UMR-BIPAR. In addition, the strong scientific collaboration/connection with Dr. A. Cabezas-Cruz (INRAE), who is an internationally recognized expert in tick-pathogen interactions, was the crucial point in creation of this research direction, we together named NeuroPaTick (Neuro-neurophysiology, Pa -pathogens, Tick – ticks). This merging process of tick physiology and the pathogen they transmit resulted in successful research grant "*Neuronal Basis for Tick-Pathogen Interactions*", Île-de-France DIM-One Health (103.600 euros, PI: Ladislav Šimo).

Specifically, the project aimed to examine the relationship of *Anaplasma phagocytophilum* with tick central nervous system, the synganglion. *A. phagocytophilum* is one of the most widespread tick-borne pathogens in animals in Europe, and also affects also human health (Kocan et al., 2015). Current control and prevention strategies against the transmission of this pathogen mostly rely on the reduction of tick populations by chemical acaricides and only antibiotic-based treatments of clinical cases have been effective. Although the capacity of A. phagocytophilum to manipulate cellular processes is well established (Ayllón et al., 2015; Cabezas-Cruz et al., 2017; Lin et al., 2011), the interaction of specific signaling molecules within the tick central nervous system, i.e., the synganglion, is overlooked. In the proposed research, we aim to identify the tick signaling molecules (neuropeptides), related to *A. phagocytophilum* infection and study their function in relation to the transmission or acquisition of this pathogen. Such an approach is expected to lead to the development of environmentally benign strategies for the control of this important tick-borne pathogen. Tick neuropeptidergic system is an ideal target for the development of novel control measures for various tick-pathogen interactions with a strong potential to lead to the reduction of their negative impacts on human and animal health. The major questions we aim to address are: (i) Have the tick-borne pathogens the capacity to manipulate tick signaling molecules and subsequently modulate tick physiology or behavior? (ii) Do the pathogen and/or the tick benefit from such modulation of tick physiology or behavior? And most importantly (iii) How can tick signaling pathways be at the basis of devising better control strategies for ticks and subsequently the pathogens they transmit? The project strongly benefits from the collaboration of the INRA proteomic platform Pappso (UMR-MICALIS, INRA centre Jouy en Josas), which is the official partner of the project and this collaboration already resulted in the massive amount of preliminary data in the field of quantitative peptidomics.

For this study I supervised a postdoc Dr. Lourdes Mateos-Hernandez and our pilot study has been published in *Pathogens* Journal (Mateos-Hernández et al., 2021). The specific aim of this project was establishment of an *in vitro* tool for studying interaction of tick-borne pathogens with neural components of tick physiology. *I. scapularis* embryonic ISE6 cell line is the most frequently used tool for propagation of various tick-borne pathogens (Munderloh et al., 2009, 1996). Although the *I. scapularis* ISE6 cell line was established almost three decades ago (Munderloh et al., 1996); its tissue origin, or biological background, remains ambiguous. A recent study recently suggested that the cell line is a mixture of different cells with the predominant-neuron like type (Oliver et al., 2015). Therefore we employed a massive in silico BLAST search of ISE6 databases to determine if the cells express the neuropeptides molecules and thus can be considered aw neuropeptides neurons. Indeed among 38 neuropeptides

genes, 37 neuropeptide transcripts were confirmed to be expressed in the ISE6 Sequence Read Archive (SRA) databases (Table 1. in Fig. 22). Subsequently we selected one neuropeptide, tachykinin-related peptide (TRP) and test if infection of the ISE6cells with different *Anaplasma* strain affect its expression (Fig. 22A-M). Our study shows that *trp* levels in ISE6 cells are differentially regulated in response to infection by different *A. phagocytophilum* strains with specificities for bovine BV49 (Silaghi et al., 2018), ovine NV2Os (Stuen et al., 2002), or human NY18 (Asanovich et al., 1997) hosts (Fig. 22A). Although the precise molecular mechanism(s) by which particular *A. phagocytophilum* strains interact with TRP is unknown, our data suggest that *A. phagocytophilum* modulates TRP transcript levels in a strain-specific manner (Fig. 22A).



Neuropeptides in ISE6 cell line

Neuropeptide name	Computed annotation	Scaffold	mRNA (SRA)
Adipokinetic hormone/corazonine-related peptide, (ACP)	XR_003917229	PKSA02006111.1	✓
Achatin-like (GFGE)	XM_029988349	PKSA02000030.1	✓
Allatostatin CC	ISCI001408	PKSA02005862.1	✓
Allatotropin	ISCI017791	PKSA02000866.1	✓
Arginine-vasopressin-like peptide (Inotocin)	XM_029985633[1]	PKSA02000317.1; PKSA02014421.1	✓
Bursicon alpha	ISCI004617	PKSA02001242.1	✓
Bursicon beta	ISCI004618	PKSA02001242.1	✓
Calcitonin-like diuretic hormone 34a	ISCI020490[1]	PKSA02012946.1; PKSA02003019.1	✓
Calcitonin-like diuretic hormone 34b	ISCI009341	PKSA02005071.1	✓
CCHamide-1	ISCI013057	PKSA02001506.1	✓
Corticotropin-releasing factor-related diuretic hormone	ISCI007845[1]	PKSA02003125.1; PKSA02006996.1	✓
Corazonin	ISCI014429	PKSA02006111.1	✓
Crustacean cardioactive peptide	ISCI010619	PKSA02006111.1	✓
Crustacean hyperglycaemic hormone/related ion transport peptide (CHH/ITP) isoform a	ISCI023228[1]	PKSA02003886.1; PKSA02006116.1	✓
Crustacean hyperglycaemic hormone/related ion transport peptide (CHH/ITP) isoform b	XM_029989718[1]	PKSA02003886.1; PKSA02006116.1	✓
Eclosion hormone	ISCI001941	PKSA02005732.1	✓
EFLamide	ISCW014582[1]	PKSA02010407.1; PKSA02009519.1	✓
Elevenin	AXL48134.1[1,2]	PKSA02008257.1; PKSA02016953.1 PKSA02007065.1; PKSA02009048.1	✓
FGLa-related allatostatin (Allatostatin A)	ISCI022939[1]	PKSA02013181.1; PKSA02004535.1	✓
Glycoprotein A2	XM_029991759[1]	PKSA02013868.1; PKSA02004257.1	✓
Glycoprotein B5	ISCI010926[1]	PKSA02004257.1; PKSA02012429.1	✓
Insulin-related peptide 1	ISCI002549[1]	PKSA02002782.1; PKSA02000328.1	✓
Insulin-related peptide 2	ISCW020331	PKSA02008623.1; PKSA02013879.1	✓
PISCF-related allatostatin (Allatostatin C)	ISCI001803	PKSA02005862.1	✓
Myoinhibitory peptide (Allatostatin B)	ISCI017595[1]	PKSA02004180.1; PKSA02003484.1	✓
Kinin	ISCI024200	PKSA02005591.1	✓
Natalisin	ISCI021632	PKSA02002188.1	ND
Neuroparsin	XM_029993215	PKSA02004554.1	✓
Orcokinin	ISCI010518	PKSA02003264.1	✓
Proctolin	ISCI005701[1,2]	PKSA02003526.1; PKSA02018840.1 PKSA02018839.1	✓
Prothoracicotropic hormone-like (Trunk)	ISCI001809	PKSA02005517.1	✓
Pyrokinin	ISCI019582	PKSA02005732.1	✓
RYamide	ISCI005825	PKSA02000030.1	✓
SIFamide	ISCI022950	PKSA02005457.1	✓
Short neuropeptide F	ISCI007409	PKSA02002025.1	✓
Sulfakinin	XM_029979447	PKSA02001743.1	✓
Tachykinin related peptide (TRP)	**ISCI008383***	**PKSA02005591.1**	✓
Trissin	ISCI011258	PKSA02005519.1	✓

Figure 22. Left panel: Table 1. Neuropeptide genes identified in ISE6 genome sequence and transcript databases. [1]Possible allelic forms of two scaffolds. [2]The gene likely spans multiple scaffolds (and multiple predictions); *Incorrect, partially predicted transcript; ND—not detected. Note that XM, XR, and AXL predicted transcripts are from NCBI databases of ISE6 cell and were not detected in VectorBase ISE6 datasets. Sequence Read Archive (SRA) corresponds to Illumina transcript reads. **Right panel:** (A-M) Expression of ISE6 tachykinin related peptide (TRP) in response to *A. phagocytophilum* infection. (**A**) Fold changes in the transcript levels of TRP the ISE6 cells (y-axis) infected ~80% with three different strains (NY18, NV2Os, and BV49) of *A. phagocytophilum* (x-axis). The asterisk (*) indicates the comparison of the standard mean error to the uninfected ISE6 value using a one-way Student's t-test. (**B–G**) Immunocytochemistry (ICC) analyses of *I. scapularis* TRP in uninfected and NV2Os-infected ISE6 cells. (**B,C**) TRP-like IR (green, arrows) in uninfected cells. The TRP-like IR (green; arrows) for ~60% (**D,E**) and ~80% (**F,G**) infection of the cells with *A. phagocytophilum* NV2Os strain. (**H–M**) Negative control staining (i.e., only the secondary antibody was used) for uninfected as well as ~60% and ~80% infected cells. Blue labeling are the (DAPI) staining. Scale bars are 10 µm. Wholemount *trp in situ* hybridization (ISH) and TRP immunohistochemistry (IHC) in synganglion from *I. scapularis*. (**N**) ISH staining with TRP antisense probe and schematic drawing in (**O**). In the schema (O), ISH-stained dorsal neurons are colored in black and ventral neurons are colored in red. (**P**) TRP-like IR (green) in *I. scapularis* synganglion. Arrowheads show the axonal projections originating from Pd_1DL neurons. (**R**) Different Z-stack positions of the synganglion highlighting the TRP-like IR (green) neurons. (**S**) Lateral side of the synganglion highlighting the TRP-like IR (green) lateral nerve (LN) and associated lateral segmental organs (arrows). (**T,U**) Magnified view of the lateral segmental organs (LSO). Arrows in U show the TRP-like IR in cells of LSO, while arrowheads show the TRP-like axon within the hemal nerve 1 (HN1), running along the LSO. Dotted lines in U show the boundaries of the different LSO cells. (**V**) Lateral posterior part of the synganglion with associated nerves. Arrowheads show the TRP-like IR axons in four pairs of opisthosomal nerves (OsN), (**Z**) Schematic drawing of *I. scapularis* synganglion summarizing all detected TRP-like IR (green) including neurons and axonal projections. Hemal nerves 1-3 (HN1-3), periganglionic sheath (PgS). Asterisks indicate TRP-like IR in axon terminals on dorsal surface of PgS. Scale bar for A–E is 50 µm, for F, G is 10 µm and for H, I is 50 µm. (Mateos-Hernandéz et al., 2021)

In parallel we examined the expression of TRP in tick synganglion in both mRNA and peptide level (Fig. 22N-Z). Considering that neuropeptides are regulators of all tick physiological processes and pathogens modulate tick physiology (Schoofs et al., 2017), our study suggests that ISE6 cells are an effective *in vitro* archetype for investigating TBP interactions with vital elements (i.e., neuropeptides) of the tick synganglion. Therefore, investigating tick–pathogen interactions by enlisting parallel, yet similar, cell-types (i.e., ISE6 cells and tick synganglion) may contribute to advancing tick control strategies to prevent TBP transmission. Our current study is a potential stepping stone for *in vivo* experiments to further examine the neuronal basis of tick–pathogen interactions. We anticipate that identifying the crucial neuronal components in tick–pathogen interactions will present key targets for developing novel tick management strategies applicable to a broad spectrum of tick-borne pathogens. In addition, such an approach has a potential to replace classical acaricides with environmentally benign drugs, targeting exclusively tick vital functions with high specificity.

As I continued to work with tick signaling system as wells as tick-borne pathogens I was invited to write together with Dr. Daniel Soenshine (Old Dominion University, USA) a book chapter: *Biology and Molecular Biology of Ixodes scapularis,* in a prestigious book named *Lyme Disease and Relapsing Fever Spirochetes: Genomics, Molecular Biology, Host Interactions and Disease Pathogenesis* (Sonenshine and Šimo, 2021) (see right site of this paragraph).

3.4.4 Exploring the neural signaling pathways in *Trichinella spiralis*

This pioneer project is developed in collaboration with Dr. Gregory Karadjian (ANSES, UMR-BIPAR). The goal of the project is to investigate for the first time the neuropeptides and neuropeptide and neurotransmitter transmembrane receptors in the food-borne parasite helminth *Trichinella spiralis*. The recent genome project of *T. spiralis* revealed a number of GPCR receptors, indicating the functional neural processes in this medically important helminth. Our project supervising one Master 2 student Caina Ning (University Paris-Saclay), aim to employ the bioinformatics, biochemistry and molecular biology approaches to examine the presence and spatial localization of molecules involved in the *Trichinella* signaling pathways. In addition, the selected neuropeptides and GPCRs receptors are going to be molecularly characterize and functional tested in our heterologous expression systems. We predict that exploration of *Trichinella* neural signaling may shed a completely new light on the *Trichinella* parasitic lifestyle and uncover the key factors underling the helminth-host interactions. In the long term this pioneer study is expected to lead to the identification of vulnerable spots in *Trichinella* physiology, which can be advantageous for the development of effective control measures of this parasite.

This project occupies a unique space in the French research space by aiming for the first time to investigate nematodes neuropeptidergic system in France.

3.4.5 New insights regarding tick co-infections?

In 2021 the MiTick team, I am leading in UMR-BIPAR (please see the organigramme below, part 5.) got awarded by a collective funding from LabEx, IBEID Pasteur Institute 2021-2024. The project named *New insights regarding tick co-infections?* is divided to three parts:

Part 1.) Tick co-infections: in ticks and in mammals (PI: Sara Moutailler, ANSES), Part 2.) Impact of coinfections on tick microbiome and tick physiology (PI: Alejandro Cabezas-Cruz, INRAE), Part 3.) Impact of anti-tick microbiota and anti-gut GPCRs vaccines (PI: Ladislav Šimo, INRAE). The project is expected to start in May 2021 with the overall funding of 635 000 euros.

Co-infection in humans and animals might enhance disease severity as has been reported for concurrent babesiosis and Lyme disease (Grunwaldt et al., 1983), and may also have significant consequences in terms of tick-borne disease treatment and diagnosis (Moutailler et al., 2016). In contrast, little is known regarding synergistic or antagonistic associations that could exist between pathogens in ticks or in animals during co-infection, and thus need to be studied. Here we aim to employ *in vivo* and *in vitro* models to study the impact of co-infections in pathogen fitness, tick microbiome and tick physiology. Such approach is expected to clarify pathogen transmission mechanisms from tick to tick or tick to host during the co-feeding process in the context of co-infection versus a single infection. We strongly believe that elucidation of such aspects of tick-pathogens interactions will uncover key targets for developing novel tick management strategies, which could be applicable to a broad spectrum of different tick-host pathogen interactions.

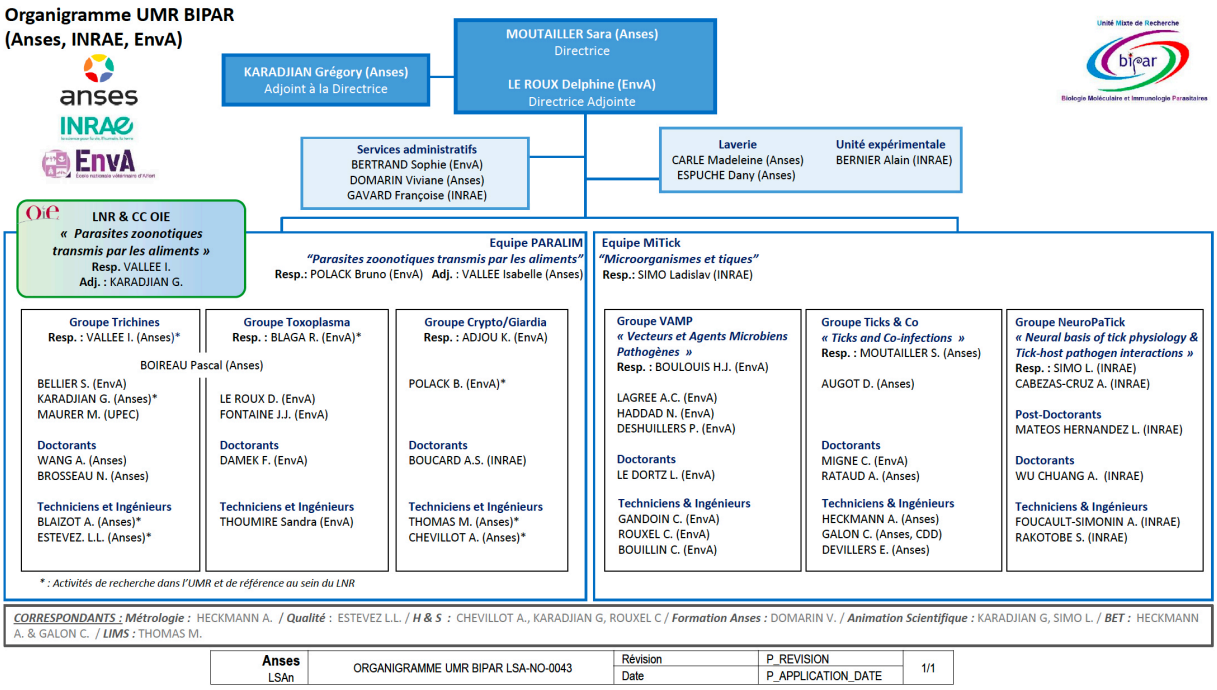
My specific input in the project is to identified bacteria sensing cell surface GPCR receptors in *I. ricinus* tick. Identification of specific tick borne pathogens/microbiota-encoded GPCRs ligands will lead to examination of their impact on local and/or systemic tick physiology. We predict that hundreds of bioactive metabolites produced by tick microbiota and pathogens agonize specific neuropeptides or neurotransmitters transmembrane receptors to alter various aspects of tick physiology. In addition we suggest that individual tick-borne pathogens influence the tick microbiota composition and subsequently their metabolome. Thus the identification of such bioactive metabolites influencing particular GPCR-driven signal transduction at different tick body sites, will serve as a baseline for deeper understanding of multiple microbiota/pathogens-tick interactions. In addition, such an approach has a strong potential to elucidate specific points in tick physiology manipulated by microbiota/pathogens for facilitation of their dissemination, survival and subsequently (co)transmission. Here I will co-supervise a postdoctoral researcher for two years and one PhD student starting in 2022.

4. CONCLUSION

Ticks have existed for hundreds of millions of years, and their ability to survive the challenges of multiple episodes of dramatic climatic change firmly place them as highly adaptable and evolutionarily successful creatures. Tick populations are booming, and there is every reason to expect that tick bites will become an escalating problem in years to come. For this reason, it is entirely reasonable to see ticks and tick-borne pathogens as "ticking time bombs", with an ever-increasing potential to explode in our changing climate. Due the rapidly escalating frequency of tick attacks, these medically important arthropods have lately attracted a great deal of attention from the world's scientific community. Current tick management methods are clearly inadequate and the key factors driving the emergence of tick-borne diseases are poorly defined. To address this problem, a multidisciplinary approach with input from scientists from various complementary fields is urgently required.

During my professional career path I have been involved in the investigation of a highly unique research field, the neural signaling pathways in hard ticks. Special attention has been paid on the interactions of tick central nervous system with their SG. The discoveries I made in this field, served as a basis to obtaining several comprehensive research grants across USA and Europe and let numerous young generation of scientist to accelerate in their professional carriers development. In addition my scientific achievements allowed me to obtain PI position in France and establish a core laboratory (www.neuropatick.com) in UMR-BIPAR, focusing on the various aspect of tick neurophysiology. The recent inclusion of tick-borne pathogens topic to my research program let to create an entirely novel research discipline in the field named: neural basis of tick pathogen interaction. Currently, not one similar tick research program exists, neither in France, nor in the rest of the world thus, our activities greatly enrich tick research at both national and international levels.

5. FUNCTIONAL ORGANIGRAM OF UMR-BIPAR



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