

Processing of odour mixture complexity in newborn rabbits

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Ca^{2+} concentration for half-maximal current activation was 4.5 μM at -50 mV and 2.6 μM at +50 mV, while the Hill coefficient was >1. For some mutants the same analysis revealed differences in Ca^{2+}- and/or voltage-dependence.

These preliminary results indicate that the region rich in glutamic acid residues located the first putative intracellular loop could be involved in Ca^{2+}- and voltage-dependence of the TMEM16B channel.

Bioluminescence Resonance Energy Transfer studies demonstrate Constitutive and Specific Homo-oligomerization of the Human Olfactory Receptor OR1740, a Rhodopsin-like GPCR

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Olfaction confers mammals the ability to perceive a myriad of odorants. Olfactory clues represent an essential survival tool for food searching, avoidance of danger and reproduction. The olfactory receptors (ORs) belong to the large super-family of G protein coupled receptors (GPCRs). GPCRs have been documented to form oligomers thought to play important roles in receptor trafficking to the cell membrane, ligand binding, G protein coupling, and intracellular signaling. To date, no study has thoroughly elucidated mammalian ORs dimerization, even though heterodimerization of ORs was reported in rodents and insects (Hague et al, 2004; Neuhaus et al, 2005; Bush et al, 2007). The relationship between a GPCR activation and its association/conformation state remains unknown.

In a previous paper (Vidic et al, 2008), the bell-shaped dose-response curve observed for receptor interaction with odorants in the absence of OBPs (odorant binding proteins) prompted us to propose a model for OR-OBP-odorant ligand interaction. Our model is based on two hypotheses, the association of ORs into homodimers, and the competitive binding of odorant and OBP to the receptor. On the one hand, we elucidated the specific role of OBP for maintaining OR activity at high odorant concentration (Vidic et al, 2008). On the other hand, investigation of the association state of ORs was crucial to fully validate the adequacy of our whole mechanistic model.

Therefore, the human OR1740 receptor, heterologously expressed in *S. cerevisiae,* was again used to address whether mammalian ORs exist as homo-oligomers and whether this phenomenon may be involved in receptor signaling upon odorant ligand activation. Co-immunoprecipitation and bioluminescence resonance energy transfer (BRET) approaches were performed to demonstrate that the OR1740 receptor constitutively homo-dimerizes. Using membrane subfractions preparations, this constitutive homo-dimerization was shown to occur early in the biosynthesis pathway, in the endoplasmic reticulum (ER). Furthermore, agonist binding induced a modulation in BRET levels, suggesting a conformational rearrangement of the receptor related to its activation/signaling state. The bell-shaped curve observed for the BRET variation as a function of odorant ligand concentration corroborates our previous studies (Minic et al, 2005; Sanz et al, 2005; Vidic et al, 2008).

For the first time, our results unambiguously show that mammalian ORs can self-associate into dimers early in the ER, and are constitutively expressed at the plasma membrane. This study also demonstrates that ligand-mediated conformational change can promote an inactive state of the OR dimers at high ligand concentration. In conclusion, the existence of the OR homo-dimers supports and validates our model based on the tripartite OBP-odorant-OR partnership (Vidic et al, 2008).

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Odorant dependent secretion on the antennal surface in the cockroach, *Periplaneta americana*

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Temporal resolution is an important feature of olfactory perception allowing orientation towards an odor source. It substantially depends on stimulus inactivation rate. Until now, only the mechanisms of enzymatic degradation of pheromones were discovered and their role in source localization and mate finding was suggested.

Present study describes the odorant dependent liquid secretion onto antennal surface in response to plant-derived odors in American cockroach. Optical registration of odor stimulated antennae discovered the layer of clear liquid which was absent in non-stimulated or ambient air stimulated antennae. Long alcohol-sensitive sensilla which lack pores on the basal parts of their shafts did not change their responses over a 100 min of periodic stimulation with an odorant. Medium sized sex pheromone sensitive sensilla decreased their firing slightly. Decrease in action potential rates of short alcohol-sensitive sensilla in response to an appropriate odorant with time corresponded to the liquid accumulation. The mechanical blockage of pores at the basal parts of sensilla with this secretion is likely to be responsible for the effect.

The main questions arose are 1) what is the chemical composition of the secretion, and 2) whether brain is involved into mediating the signal of odorant presence. Freely behaving insects enhance antennal grooming when exposed to the olfactory stimuli, supposedly cleaning out the surplus of secretion with odorant dissolved in it. It is interesting to note that frequency of the leg grooming did not change under exposition to odor stimuli. Thus the displacement behavior as a reaction to odor is the less plausible explanation. The mechanism of odorant elimination described here seems to be non-specific and does not depend on biological significance of the stimulus.

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Humans have limited abilities to elementally process odour mixtures. In a mixture including less than 16 odorants we do not recognize more than 4 components, and in a mixture including more than 16 odorants we do not recognize any of them. At a certain level of complexity, mixtures are then configurally processed in humans[1].

Here, we asked for the level of complexity required to induce a configural processing of odour mixture in another species, the European rabbit, at an important period of development, the neonatal period. We used a 6 components mixture which forms Red Cordial (RC) blending in humans[2]. We have previously shown that rabbit pups efficiently extract all the elements from this complex mixture after learning RC mixture. However, they do not respond to the RC mixture after learning one component, which suggests that they do not recognize the learned component within the mixture[3]. One may hypothesized that perceptual blending occurs in this mixture and led, inr rabbits as in humans, to the emergence of an odour quality different from the quality carried by each component. Alternatively, one may also suggest that a 6 components mixture, whatever the mixture, contains too much un-learned information to allow the extraction of previously learned fractional information. To evaluate such possibilities we conditioned[4,5], in a first experiment, 55 one-day-old rabbits to one component of the RC mixture. We tested, 24h later, their oral response[4,5] to the mixture deleted of one component (5 possible quinary mixtures). Between 83 and 94% of the rabbits responded to the quinary mixtures. Moreover, in a second experiment, we began to evaluate the responsiveness of pups to the RC mixture but including various ratios of 2 of the 6 components or all of the 6 components. Final results will validate one or the other hypothesis and shed some light on whether the high number of information or the intrinsic blending properties of a mixture lead to a configural processing.

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Banana odor blend processing in the olfactory circuitry of *drosophila melanogaster* – Linking gas-chromatography with optical imaging techniques

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Natural odors occur in complex blends consisting of many different components. Such a blend might be characterized by a single key compound (a single dominant compound) or by several compounds. The central question of this study was if a blend including several key compounds is characterized by the mere sum of these compounds or by non-linear compound interactions.

We addressed this question by investigating physiological responses to single odor compounds and the complete odor blend emitted from a banana. These fruits are one of the most favored breeding places and a highly attractive food source for the fruit fly, *Drosophila melanogaster*. In these flies olfactory sensory neurons (OSN) convey odor information from the antenna and the maxillary palps to the antennal lobes (ALs). Neurons expressing the same type of receptor proteins converge into spherical AL sub-structures called olfactory glomeruli. Here, OSNs synapse onto projection neurons (PN) carrying odor information to higher brain structures and onto local interneurons (LN) that synapse onto neurons of other glomeruli providing a network with modulatory capacity. The concert of active glomeruli in the AL is odor compound and odor blend specific. How the network processes simultaneous input regarding several different odor compounds to form a specific activity pattern is poorly understood.

Using coupled gas-chromatography / calcium imaging techniques active banana odor compounds were characterized and their activity patterns in the olfactory system of the fruit fly were described. Comparison between calcium dynamics among OSN input and PN output channels revealed significant differences due to network processing. Comparisons between single compound and whole banana blend stimulation responses revealed blend interactions only in few glomeruli.

We could observe multiple processing effects revealing that the processing of a natural banana odor blend is not linear. Network processes altered component representations and changed their similarity to the blend. The center of AL activity, glomerulus DM2, was most strongly activated by most blend components, both in excitatory and inhibitory interactions. The GC-imaging method thus allows detailed investigations of the olfactory network and will be a highly useful tool in future investigation of insect-insect and insect-plant interactions.

Candidate chemosensory ionotropic receptors in a lepidoptera

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Recently, a new family of candidate chemosensory receptors was discovered in *Drosophila melanogaster* (Benton et al, 2009). These receptors are related to ionotropic glutamate receptors (iGluRs) and thus were called ionotropic receptors (IRs) but, phylogenetically, they do not belong to the iGluR family.

Through Blast analyses of an expressed sequenced tag library prepared from male antennae of the noctuid moth *Spodoptera littoralis*, we identified twelve unigenes encoding proteins related to *D. melanogaster* and *Bombyx mori* IRs (V. Croset and R. Benton, personal communication). Their full length sequences were obtained and their expression patterns were determined though RT-PCR in different tissues and according to the developmental stages. Eight

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