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# ABSTRACTS

## Abstracts from the XXIInd Congress of the European Chemoreception Research Organization, ECRO 2013, 27-29 August 2013, Leuven, Belgium

### #101 Deconstructing smell

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### #102 Distinct functional roles of tufted cell and mitral cell inputs in odor information processing in the olfactory cortex

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In the mammalian olfactory system, odor signals are conveyed from olfactory bulb to olfactory cortex by two types of projection neurons, tufted cells and mitral cells. Recent studies indicate that tufted cells and mitral cells differ in function and the pattern of axonal projection to the olfactory cortex. Odor responses of tufted cells show lower concentration threshold, shorter onset latency from the inhalation onset, and higher firing frequency compared with those of mitral cells. Most noteworthy is the observation that tufted cells and mitral cells differ in signal timing within a respiration cycle. In addition, tufted cells and mitral cells show strikingly different pattern of axonal projection to areas of the olfactory cortex. Individual tufted cells project axons to focal targets in anterior areas of the olfactory cortex, whereas individual mitral cells dispersedly project to nearly all areas of the olfactory cortex. In this symposium, I would like to talk our model in which tufted cell inputs and mitral cell inputs play distinct functional roles in olfactory information processing in the neuronal circuits of the olfactory cortex and higher olfactory centers.

### #103 Activation of adult-born olfactory bulb interneurons facilitates learning and memory

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The olfactory brain is flexible, from cognitive areas all the way down to the peripheral areas in which sensory information is encoded so as to facilitate the subsequent extraction of relevant information. It is becoming increasingly clear that olfactory adaptability operates at the level of neural circuits. In the adult olfactory bulb circuit, new neurons are constitutively recruited throughout life and form an integral part of the normal functional network. This presentation focuses on the functional issues linked to the neurogenic plasticity of the sense of smell. After outlining the processes of adult neurogenesis in the olfactory system and discussing their regulation by various factors, I will explore the possible functional role of newly-formed neurons in the host olfactory circuits. Concentrating exclusively on mammalian systems, I will demonstrate throughout this presentation that adult neurogenesis is a plastic mechanism by which the olfactory bulb performance can be optimized. Special emphasis will be given on top-down processes.

### #104 Piriform cortical function in context

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The piriform cortex receives sensory input directly from the olfactory bulb, and in turn projects to a variety of forebrain regions including prefrontal cortex, thalamus, and several limbic system components. It has been hypothesized that the piriform cortex is critical in the synthesis of odorant feature input to form experience-dependent synthetic odor objects important for perception. However, this cortical synthesis of olfactory bulb activity occurs in the context of feedback from many of the higher order targets of piriform projections. This feedback may provide a multisensory, memorial, and hedonic context that shapes cortical odor coding and perception. Our lab has been using electrophysiological, behavioral, neurochemical and optogenetic techniques to explore the role of top-down feedback on piriform cortical function and odor perception in rats and mice. Here, I will discuss new results regarding the role of the lateral entorhinal cortex (LEC) and basolateral amygdala (BLA) on piriform function and odor perception. These inputs modulate the temporal structure of piriform cortex odor evoked activity, and play an important role in shaping olfactory acuity.

## #105 Agonist-independent GPCR activity regulates axon targeting of olfactory sensory neurons

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G-protein coupled receptors (GPCRs) are known to possess two different conformations, active and inactive, and spontaneously alternate between the two in the absence of agonists and inverse agonists. For different odorant receptor (OR) species, variable but unique levels of baseline activities have been observed. However, the agonist-independent baseline activity had long been considered to be noise created by GPCRs, and its functional role was not fully appreciated. Here, we analyzed the agonist-independent GPCR activity for its possible role in receptor-instructed axonal projection. We generated transgenic mice expressing activity mutants of the β2-adrenergic receptor, a well-characterized GPCR with the highest homology to ORs. We found that mutants with altered agonist-independent activity changed the transcription levels of axon guidance molecules, thus, causing shifts in glomerular locations. Knockout and in vitro experiments demonstrated that a stimulatory G protein (Gs), but not Golf, is responsible for mediating the agonist-independent OR activity. We conclude that the equilibrium of conformational transitions set by each OR is the major determinant of expression levels of axon-targeting molecules.

## #106 SeeDB: a simple and morphology-preserving optical clearing agent for neuronal circuit reconstruction

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We report a water-based optical clearing agent, SeeDB, which clears fixed brain samples in a few days without quenching many types of fluorescent dyes, including fluorescent proteins and lipophilic neuronal tracers. Our method maintained a constant sample volume during the clearing procedure, an important factor for keeping cellular morphology intact, and facilitated the quantitative reconstruction of neuronal circuits. Combined with two-photon microscopy and an optimized objective lens, we were able to image the mouse brain from the dorsal to the ventral side. We used SeeDB to describe the near-complete wiring diagram of sister mitral cells associated with a common glomerulus in the mouse olfactory bulb. We found the diversity of dendrite wiring patterns among sister mitral cells, and our results provide an anatomical basis for non-redundant odor coding by these neurons. Our simple and efficient method is useful for imaging intact morphological architecture at large scales in both the adult and developing brains.

## #107 Two chemoreceptors in one TRP channel

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Sensory neurons detect chemical stimuli through projections in the skin and mucosa, where several transient receptor potential (TRP) channels act as primary chemosensors. Functional TRP channels are tetramers, and it is generally accepted that binding of distinct chemical ligands causes the opening of a single central cation-conducting pore. Contrary to this view, we provide evidence for a second cation permeation pathway in a sensory TRP channel. Opening of this alternative pathway enhances excitation of sensory neurons and thereby exacerbates pain. Our findings indicate that a single sensory TRP channel can encompass two distinct ionotropic chemoreceptors, which may have important ramifications for TRP channel function and pharmacology.

## #108 TRPA1 channels are neuronal chemosensors of bacterial endotoxins

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TRPA1 is a member of the transient receptor potential (TRP) family of cationic channels, expressed in nociceptive sensory terminals of primary sensory neurons. Activation of TRPA1 produces pain, neuropeptide release and inflammation. TRPA1 has emerged as a key chemosensor for many natural and industrial chemical irritants.Gram(-) bacterial infections are generally accompanied by inflammation and pain. These symptoms are generally attributed to sensitization of nociceptors by inflammatory mediators released by immune cells through activation of the Toll-like-receptor 4 (TLR4) signaling pathway by lipopolysaccharide (LPS), a toxic byproduct of bacterial lyses. Unexpectedly, we found that LPS exerts fast, membrane delimited, excitatory actions on TRPA1. Moreover, we found that pain and acute pathophysiological vascular reactions, including neurogenic inflammation (CGRP release) caused by LPS are primarily dependent on TRPA1 channel activation in nociceptor terminals. The identification of TRPA1 as molecular

determinant of LPS effects opens novel avenues for the treatment of symptoms caused by Gram(-) bacterial infections and offers new insights into the pathogenesis of pain and neurovascular responses during bacterial infections.

## #109 TRP channels, gustatory receptors, and the interrelated evolution of chemical and thermal detection

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Chemical and thermal detection in animals frequently involves cation channels of the transient receptor potential (TRP) family. We study the function and evolution of such sensory detectors, focusing on TRPA1, an ion channel well known for its role in sensing wasabi, tear gas, and other noxious chemical stimuli. Recent work investigating the evolutionary origins of TRPA1-mediated sensory detection, uncovered through analysis of Drosophila TRPA1's ability to respond to both chemical and thermal stimuli, will be presented. These studies have revealed how (and potentially why) TRPA1's properties have been modulated over the course of evolution to endow different cells and different species with different behaviors. In addition, recent work discovering a central role for a gustatory receptor in warmth detection in Drosophila will be discussed.

## #110 Local anesthetic action of the TRPA1 agonist cinnamaldehyde

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Cinnamaldehyde (CA) is a pungent compound that has been extensively used to study the mechanisms of pain and neurogenic inflammation and as selective agonist of TRPA1, a cation channel involved in various inflammatory diseases. We have found that, in contrast to another TRPA1 agonist allyl isothiocyanate (AITC), CA triggered very weak irritation in human nasal mucosa and little aversion in mice. Cystometry recordings yielded that induced weak mouse bladder irritation. Intraplantar injections of CA elicited very little acute pain in mice. Notably, CA strongly reduced the pain responses induced by AITC and capsaicin. Recordings in mouse hippocampal brain slices and in mouse lingual nerve revealed that CA inhibits firing activity. Finally, we found that CA blocks voltage-gated sodium channels and that the strength of the block is reduced by a mutation at the local anesthetic binding site. We conclude that CA excites nociceptors through its action on TRPA1, but in parallel induces a local anesthetic effect. These findings are essential for the understanding of the chemosensory properties of CA, and prompts for a careful interpretation of the results obtained with this compound when used to study the pathophysiological role of TRPA1.

## #111 Two insulin-like peptides modulate an olfactory circuit to generate experience-dependent plasticity

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Insulin-like peptides (ILPs) regulate key physiological events, including neural plasticity. However, the mechanisms for their function in learning are largely unknown. We characterize two ILPs that play antagonistic roles in aversive olfactory learning [1]. The ILP ins-6 acts from sensory neurons ASI to facilitate learning by repressing the transcription of the ILP ins-7 in URX neurons. The URX-generated INS-7 inhibits learning by antagonizing DAF-2 insulin receptor in a postsynaptic neuron RIA. RIA plays an essential role in aversive olfactory learning and exhibits sensory-evoked global activity and locomotion-correlated local activity in axon [2, 3]. Increased ins-7 expression in URX, as a result of losing ins-6, alters RIA activity and disrupts learning. Intriguingly, ins-7 expression in URX increases when animals go through the dauer stage, a developmental arrest that can be induced by starvation, suggesting that post-dauer animals reduce learning and ILPs modulate learning in a context-dependent manner. These results reveal the circuit mechanisms whereby an ILP pathway regulates learning.1.Chen, Z. et al., Neuron 77(2013), 572-.2.Ha, H-I. et al., Neuron 68(2010), 1173-. 3.Hendricks, M. et al., Nature 487(2012), 99-.

## #112 An insulin-like peptide code for physiology

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The environmental influence on an animal's physiology is mediated by an insulin-like signaling pathway and is evident in the pleiotropic effects of the pathway. However, the underlying mechanisms for this pleiotropy are largely unknown. The genomes of many species contain multiple insulin-like peptides (ILPs), a number of which are expressed in sensory neurons and interneurons. This raises an intriguing possibility that the ILPs process different environmental information [1], giving rise to the extensive roles of insulin-like signaling in physiology and behavior. Here I will present how different members of the C. elegans ILP family regulate distinct processes in response to the environment, thereby uncovering a combinatorial code for physiology. Since the 40 C. elegans ILPs also exhibit network properties, this suggests a mechanism through which phenotypic specificity and combinatorial coding can be implemented.Reference:[1] Cornils, A., Gloeck, M., Chen, Z., Zhang, Y. & Alcedo, J. Specific insulin-like peptides encode sensory information to regulate distinct developmental processes. Development 138, 1183 - 1193 (2011).

## #113 Olfactory memory formation and forgetting in Drosophila

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Recent progress using in vivo, functional cellular imaging has facilitated the detection and analysis of olfactory memory traces in Drosophila. These memory traces are detected by increased calcium signals or altered synaptic transmission from specific neurons in response to the learned odor after conditioning by comparison to control flies. The memory traces form in different neurons in the olfactory nervous system and occupy different temporal windows after conditioning, which approximate the time windows for short-, intermediate-, and long-term behavioral memory. These memory traces will be discussed as well as how they differ when using aversive or appetitive unconditioned stimuli coupled to the same odorant conditioned stimulus. Recent progress in dissecting the molecular and cellular mechanisms for active forgetting will also be discussed.

## #114 The cells for bitter reception

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Bitter taste is versatile enabling animals to avoid some bitter compounds and ingest others with their food. The substances eliciting bitterness are recognized by the taste 2 receptor (Tas2r) family consisting of members with very broad, moderate or narrow molecular receptive ranges. Expression of subsets of these receptors but not of the full repertoire by individual chemosensory cells of the oral epithelium generates a heterogeneous population of bitter-dedicated cells. Whereas several Tas2rs appear to be always coexpressed in the same cells, some Tas2r combinations are never coexpressed. For other pairs of Tas2rs, the extent of coexpression ranges from ~30–90%. In this way, the bitter-dedicated cells demonstrate overlapping yet distinct response profiles. Accordingly, mice that lack the Tas2r131 expressing cell population show diminished avoidance of many bitter substances in brief access tests. In marked contrast, they are indistinguishable from control animals regarding their licking responses to denatonium benzoate and, unlike control mice, they do not avoid papaverin. The data propose that the gustatory system for bitterness is designed to enable animals to variably cope with the innumerable bitter substances.

## #115 Crosstalk and backtalk in mammalian taste buds: cell-cell communication and signal processing

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Taste buds represent a community of different cells. These cells fall into one of three broad categories: Type I, or glial-like cells whose functions are still being debated; Type II or Receptor cells, which express proteins associated with taste transduction for sweet, umami, or bitter compounds; and Type III or Presynaptic cells, which express synaptic proteins and have ultrastructural features of synapses. Taste cells communicate with afferent sensory fibers and with each other during taste reception by releasing neurotransmitters, including ATP, acetylcholine, GABA, norepinephrine, and serotonin. We have been identifying these transmitters and investigating their postsynaptic targets by using cellular biosensors—CHO cells stably expressing high affinity transmitter receptors that make the cells highly sensitive to transmitters released by taste buds and taste cells. The findings

make it clear that during taste excitation, a number of these transmitters are released and execute feedforward as well as feedback signaling, both excitatory and inhibitory. We are attempting to decipher how this crosstalk between cells contributes to taste coding and transfer of information from taste buds to gustatory sensory afferent fibers.

## #116 Involvement of multiple populations of taste cells and afferent neurons in umami taste detection

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Umami is elicited by glutamate (MSG) and 5'-ribonucleotides such as IMP and GMP. Recent molecular biological studies have identified candidates for umami receptor, including the heterodimer T1R1+T1R3, and taste-mGluR4, truncated-mGluR1, brain-mGluR1 and brain-mGluR4. In heterologous expression systems, human T1R1+T1R3 is preferentially activated by MSG, whereas mouse T1R1+T1R3 is activated by various amino acids, and both human and mouse T1R1+T1R3 show the synergism of umami responses by addition of IMP. However, to date, the physiological roles in umami taste perception of each of these receptors are unclear. T1R3-KO mice studies showed that behavioral preference for and neural responses to MSG and/or MSG+IMP reduced or abolished. Several other reports demonstrated residual neural, behavioral, and taste cell responses to MSG in T1R3-KO mice suggesting involvement of receptors in addition to T1R1+T1R3 in umami detection. In my talk, I will present our recent findings further suggesting multiple receptors and coding systems for umami taste, including that responses evoked by different umami stimuli could be recorded in separate populations of taste cells or afferent neurons and that umami taste persists in the absence of T1R1.

## #117 Molecular dissection of human bitter taste receptor activation

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For the detection of the countless bitter compounds nature employs its perhaps most versatile tools, G protein-coupled receptors. The low number of only 25 human bitter taste receptors (TAS2Rs) compared to the number of bitter compounds requires that each TAS2R should recognize many rather than few agonists. The deorphanization of 21 TAS2Rs revealed that this is indeed the case, however, individual TAS2Rs deviate considerably in their tuning breadths, ranging from broadly tuned "generalists" to narrowly tuned "specialists". Structure-function analyses demonstrated that even broadly tuned TAS2Rs possess only single ligand binding pockets tailored to accommodate various bitter substances at the expense of potentially higher affinities for individual agonists. Further, we demonstrated that identical agonists interact with different TAS2Rs via individual binding modi suggesting that the pharmacological versatility of TAS2Rs may even exceed the broad tuning properties evident in functional experiments. Our recent identification of natural bitter blockers indicates that the selective pressure to generate functional diversity among TAS2Rs may not be restricted to agonist recognition but also to avoid a complete blockade by antagonists.

## #118 The role of bitter taste receptors in activating mechanisms of human gastric acid secretion

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Gastric acid secretion is needed for the digestion of food compounds, but has to be well-regulated to prevent esophageal reflux syndrome and lesions of the stomach epithelium. Acid secretion is not only induced by stimuli originating in the brain or by dietary stimuli such as food volume or dietary protein, but, in addition, via non-nutritive food and beverage constituents as shown for organic acids in wine, bitter acids in beer, and caffeine in coffee. We asked the questions whether extra-oral bitter receptors are involved and whether bitter modulating compounds may reduce gastric acid secretory activity. Human gastric tumor cells (HGT-1) were challenged with caffeine or theobromine with or without bitter modulating compounds. As a result, the taste masking activity highly correlated with the ability to decrease proton secretion. Furthermore, we identified the expression of various hTAS2R bitter receptor subtypes in HGT-1 cells by RT-qPCR, and could demonstrate the involvement of hTAS2Rs in mechanisms of gastric acid secretion by knock-down experiments. These results were verified by a pilot human intervention, in which a bitter masking compound was able to reduce the caffeine-induced acid secretion.

## #119 TAS2R genotype-phenotype associations in humans: eating and evolution

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Thousands of structurally diverse compounds elicit sensations humans describe as bitter, and these sensations are mediated by receptors encoded by ~25 genes in the TAS2R family. Many but not all bitter compounds are mutagens, abortifacients, or toxins; presumably there was strong evolutionary pressure to detect and avoid ingesting these compounds. However, the TAS2Rs are also extremely polymorphic: a high number of pseudogenes in humans suggests possible relaxation of selective pressure, yet other TAS2Rs show evidence of either positive selection or balanced selection. Data from multiple laboratories indicate polymorphisms in TAS2Rs are functional, as they associate with perceptual differences in the laboratory as well as intake of bitter foods. Recent examples will be reviewed. Conceivably, balanced selection may result from a heterozygote advantage, if the alternate form of the gene increases the receptive range of the receptor enabling the detection of a new class of ligands. There are no clear examples of this to date, but recent behavioral data in vivo suggests the TAS2R9 Val187Ala allele may be the first example of an polymorphism broadening the receptive range of a receptor, although this remains to be confirmed in vitro.

## #120 Altered brain response to chemosensory stimuli in metabolic syndrome

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Metabolic syndrome involves a constellation of risk factors for cardiac and vascular disease that are also associated with obesity, diabetes and dementia in later life. Obesity has increased to epidemic proportions, with significant health consequences. The abundance of highly palatable food may contribute to obesity, particularly as people age, yet not everyone becomes obese. Physiological state (i.e., hunger and satiety) affects the processing of appetitive stimuli by changing the reward value and salience of stimuli in healthy young individuals. Here we investigated brain activation, using fMRI, in middle aged individuals with and without metabolic syndrome while they rated the pleasantness of sweet and bitter taste. We report here results that revealed differences in the relationship between brain response and obesity (BMI) as a function of hunger and satiety in the hypothalamus. We hypothesize that altered brain response to chemosensory stimuli is one underlying mechanism that contributes to the maintenance of obesity in metabolic syndrome. We thank A. Jacobson, L. Haase, and the UCSD Center for fMRI. Supported by NIH grant No. AG004085-26 from the NIA.

## #121 Extraoral TAS2Rs: impact on metabolism

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Many compounds evoke a bitter taste through activation of a family of G protein-coupled type 2 taste receptors (TAS2Rs) found in subsets of sensory cells of the gustatory epithelium. These receptors are also expressed in non-gustatory tissues where they could mediate physiologic responses to ingested or inhaled toxins or pharmaceuticals (many of which are known TAS2R agonists) or to endogenous factors. While TAS2R agonists have been shown to cause physiological responses in a number of extraoral tissues, direct evidence that these effects are TAS2R-mediated is often lacking. I will propose criteria that should be met to clearly establish a functional role for TAS2Rs in extraoral tissues. I will also present recent studies that indicate that bitter stimuli can alter thyroid hormone production through the TAS2R-dependent regulation of thyroid follicular cell function. Thus, TAS2Rs may mediate a protective response to overingestion of toxic materials and may serve as novel targets for new thyroid disease treatments. Supported by: NIH/NIDCD grant DC010110.

## #201 Epigenetic regulation of olfactory receptor expression

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The molecular mechanisms that activate and stabilize olfactory receptor (OR) transcription are, by and large, unknown. Here, we show that histone demethylase LSD1 is required for the de-silencing and subsequent transcriptional activation of an OR allele, which in turn, induces the expression of Adenylyl Cyclase 3 (Adcy3). Adcy3 then promotes the downregulation of LSD1, stabilizing, this way, the expression of the initially chosen OR allele and preventing the activation of additional OR genes. Our data reveal the first signaling component that connects OR protein function to the epigenetic regulation of OR expression, and show that LSD1, a protein with dual, co-activator and co-repressor activity, can both activate OR transcription but also induce OR switching if its expression is not promptly terminated upon OR expression.

## #202 Promoter architecture of mouse olfactory receptor genes

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Odorous chemicals are detected by the mouse main olfactory epithelium (MOE) by about 1100 types of olfactory receptors (OR) expressed by olfactory sensory neurons (OSNs). Each mature OSN is thought to express only one allele of a single OR gene. Major impediments to understand the transcriptional control of OR gene expression are the lack of a proper characterization of OR transcription start sites (TSSs) and promoters, and of regulatory transcripts at OR loci. We have applied the nanoCAGE technology to profile the transcriptome and the active promoters in the MOE. nanoCAGE analysis revealed the map and architecture of promoters for 87.5% of the mouse OR genes, as well as the expression of many novel noncoding RNAs including antisense transcripts. We identified candidate transcription factors for OR gene expression and among them confirmed by chromatin immunoprecipitation the binding of TBP, EBF1 (OLF1), and MEF2A to OR promoters. Finally, we showed that a short genomic fragment flanking the major TSS of the OR gene Olfr160 (M72) can drive OSN-specific expression in transgenic mice.

## #203 Lhx2 and Emx2 drive odorant receptor expression

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Homeodomain-like sites are functional elements of odorant receptor (OR) gene control regions. Emx2 and Lhx2 are the most likely transcription factors acting at these cis elements. To avoid the loss of mature olfactory sensory neurons (OSNs) seen in germ line knockouts, we did conditional deletion in immature or mature OSNs. Loss of Lhx2 in immature OSNs decreased 818 OR mRNAs; deletion of Emx2 gave smaller effects. Deletion of both Lhx2 and Emx2 had a combinatorial effect, reducing 973 OR mRNAs. Of 1,098 ORs tested only 16 of were insensitive to the loss of Lhx2, Emx2, or both. These effects on mRNA abundance arose from decreased frequency of expression of OR genes. DNA binding studies show direct interaction of Lhx2 and Emx2 with OR gene promoters. Conditional deletion of Lhx2 or Emx2 in mature OSNs gave results similar to deletion in immature OSNs, arguing that Lhx2 and Emx2 help drive expression of OR genes throughout the life span of OSNs. Lhx2 and Emx2 are the major homeodomain transcription factors responsible for stimulating OR gene expression. Funded by NIH R01 DC007194.

## #204 Resolving receptors: transcriptomic analysis in olfactory systems

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Olfactory and vomeronasal receptor gene coding sequences are now reasonably well annotated in the genomes of model organisms and humans. However a number of factors conspire to hinder progress in transcriptomic analysis in olfactory systems. We have developed a very deep, paired short-read RNA sequencing ("RNAseq") pipeline that can accurately record and reproducibly quantify the entire transcriptomes of olfactory and vomeronasal tissues, including the full-length transcript sequences of most (but not all) chemosensory receptors, even in the absence of a well annotated reference genome. I will first describe some of the pitfalls that can distort RNAseq data from olfactory systems, and compare and contrast this technology with other transcriptomic techniques. I will then describe some unexpected findings when we applied RNAseq to mouse olfactory tissue, and finally present comparisons in receptor expression profiles between animals of different age, sex, strain and species. We conclude from these analyses that receptor expression repertoires can vary significantly, likely as a consequence of underlying genetic variation, and discuss the implications for regulating receptor choice, evolution and function.

## #205 Odorant receptor gene choice at the SR1 receptor gene locus

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The mechanisms behind the exclusive expression of a single olfactory receptor (OR) gene per olfactory sensory neuron

(OSN) remain poorly understood. In the mouse, OSNs express only one allele of the 1.200 OR genes in the genome. We applied an OR deletion strategy (dOR paradigm) to investigate principles of OR gene choice at the SR1 receptor gene locus. The SR1 gene constitutes an interesting model because it is simultaneously expressed in OSNs of the main olfactory epithelium (MOE) and in 50% of OSNs in the septal organ (SO), allowing us to examine context- and tissue-dependent influences on OR gene choice. We observe an unprecedented biallelic expression rate of 30% at the SR1 locus in SO neurons of heterozygous dSR1 mice but a much lower rate in the MOE. Using a combination of anatomical and molecular assays, we observe that dSR1 OSNs coexpress a total of about 80 OR genes. However, the frequencies of coexpression are different for dSR1 OSNs in the SO and the MOE, suggesting that expression of the SR1 gene is regulated differently in both tissues.

## #206 A cleavable N-terminal signal peptide promotes widespread olfactory receptor surface expression in HEK293T cells

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When heterologously expressed, most olfactory receptors (ORs) are retained in the ER and degraded. Because deorphanization assays typically require OR surface expression, most ORs remain orphan receptors. To date, studies have utilized non-cleavable rhodopsin (Rho) tags and/or chaperones to improve surface expression. However, even with these tools, many ORs fail to reach the cell surface. We used a set of 15 ORs to examine the effect of a cleavable leucine-rich signal peptide sequence (Lucy tag) on OR surface expression in HEK293T cells. The addition of the Lucy tag to the N-terminus increases the number of ORs reaching the cell surface to 7 of the 15 ORs (as compared to 3/15 without Rho or Lucy). Moreover, when ORs tagged with both Lucy and Rho were co-expressed with previously reported chaperones (RTP1S, Ric8b and Gαolf), we observed surface expression for all 15 receptors examined. As expected for a signal peptide, the Lucy tag was cleaved from the mature protein and did not alter OR-ligand binding and signaling. Our studies demonstrate that widespread surface expression of ORs can be achieved in HEK293T cells, providing promise for future large-scale deorphanization studies. Funding: NIDDK

## #207 Non-redundant coding of aversive amines in the mouse olfactory system

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Chemical stimuli are represented in the main olfactory pathway by over 1,000 odorant receptors, each of which is mapped to specific glomeruli in the olfactory bulb. The Trace Amine-Associated Receptors (TAARs) are a small, additional class of evolutionarily conserved olfactory receptors whose contribution to olfactory function is not clear. My laboratory is using gene targeting, electrophysiology, optical imaging, and behavior to identify how the TAARs contribute to olfactory function in vivo. We show that the TAARs mediate high sensitivity detection of and behavioral aversion to amines and predator-derived odors. Our data indicate that individual TAAR genes can contribute significantly to innate responses to volatile amines.

## #208 A fluorescent protein fusion toolbox for anaylyzing GPCR trafficking and functionality

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Green fluorescent protein (GFP) has proven useful for the study of protein interactions and dynamics for the last twenty years. A variety of new fluorescent proteins have been developed that expand the use of available excitation spectra. We have undertaken an analysis of seven of the most useful fluorescent proteins (XFPs), Cerulean (and mCerulean3), Teal, GFP, Venus, mCherry and TagRFP657, as fusions to the archetypal G-protein coupled receptor, the β2 adrenergic receptor (β2AR). We have characterized these β2AR::XFP fusions in respect to membrane trafficking and G-protein activation. All β2AR::XFP fusions show responses indistinguishable from each other and the non-fused form after isoprenaline exposure. Our results provide a platform by which G-protein coupled receptors can be dissected for their functionality. We are currently using Odorant Receptor fusions with fluorescent proteins to study their trafficking and functionality.

## #209 Mammalian specific OR37 receptors are differentially activated by distinct odorous fatty aldehydes

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In search for biological sources of the long-chain fatty aldehydes (penta-, hexa- and heptadecanal) which we recently identified as ligands for members of the mouse odorant receptor subfamily OR37, the headspace of secretions and excretions from mice was analyzed by gas chromatography and mass spectrometry (GC/MS). In urine, skin swabs and saliva, these components were not detectable. In feces pellets a substantial amount of hexadecanal, the OR37B ligand,

was found. Accordingly, exposure of mice to feces induced an activation of the OR37B glomerulus. The amount of hexadecanal deposited with feces varied significantly, however, it was independent from the amount of feed. In many species, feces is covered with secretion from anal glands. Due to the size and the inaccessibility of these glands in mice, the headspace of anal gland secretion from dog was analyzed by GC/MS, resulting in a prominent peak for hexadecanal. Exposure of mice to anal gland secretion from dog activated the OR37B glomerulus. Altogether, the data suggest that hexadecanal, a ligand for the receptor OR37B, is produced in anal glands and deposited with feces into the environment, where it may be an important signal for chemical communication.Supported by the DFG.

## #210 Crypt neurons constitute a labeled line for odor processing in vertebrates

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Crypt neurons are a third type of olfactory sensory neurons that originally have been identified by their conspicuous morphology including the eponymous crypt of unknown significance. We show here that crypt neurons use a more restricted mode of expression than ciliated neurons, which generally follow the 'one neuron-one receptor' rule. We recently identified a novel olfactory receptor family of 6 highly conserved v1r-like ora genes. We report now that a single member of this family, ora4 is expressed in nearly all crypt neurons, whereas the other 5 ora genes are not found in this cell type. This corresponds to a "one cell type–one receptor" mode of expression. Such an expression pattern is known from the visual system, but unexpected in the sense of smell. Furthermore, we have identified a single mediodorsal glomerulus, mdg2, as target glomerulus of crypt neurons. Thus, crypt neurons form a labeled line consisting of a single sensory cell type, a single olfactory receptor and a single target glomerulus. This type of olfactory representation is reminescent of insect glomeruli specialized for pheromone detection, and indeed ligands of ORA4 appear to serve as zebrafish pheromones.

## #211 Studying neural circuits mediating fear and anxiety in zebrafish brain

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The habenula (HB) is a conserved brain region, which connects the forebrain to monoaminergic brainstem nuclei. Genetic ablation of specific HB sub-regions was shown to alter conditioned fear response in zebrafish. In order to understand how HB circuits process olfactory information, we studied the neural activity in the olfactory bulb and HB, in response to odors. Our results demonstrated that HB circuits are broadly tuned and asymmetric, with strong odor responses in the right HB. Moreover we observed that odor responses in each HB hemisphere are topographically organized, despite the non-topographic MC terminals in HB. Analysis of ongoing spontaneous activity in HB revealed that the topographic organization of HB activity reflect the favored states of HB circuitry resembling attractor networks. These HB microcircuits are spatially organized and the activities of neighboring neurons are correlated. These HB microcicuits responded to odors similarly and remained within the same functional cluster before and during odor stimulation. Finally, we demonstrate that some of these functional micro-domains within HB overlap well with the genetically described groups of HB neurons that were shown to regulate conditioned fear response.

## #212 Olfactory wiring logic in amphibians challenges the basic assumptions of the unbranched axon concept

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Olfactory receptor neurons extend axons into the olfactory bulb, where they face the challenge to integrate into existing circuitry. The consensus view is that in vertebrates individual receptor neurons project unbranched axons into one specific glomerulus of the olfactory bulb. We report here, that strikingly different from the generally assumed wiring principle in vertebrate olfactory systems, axons of single receptor neurons of Xenopus laevis regularly bifurcate, and project into more than one glomerulus. Specifically, the innervation of multiple glomeruli is present in all ontogenetic stages of this species, from the larva to the post-metamorphic frog. Also, we show that this unexpected wiring pattern is not restricted to axons of immature receptor neurons, but that it is also a feature of mature neurons of both the main and accessory olfactory system. This glomerular innervation pattern is unique among vertebrates investigated so far, and represents a new olfactory wiring strategy.[Supported by DFG-SPP/1392 and DFG-CNMPB]

## #213 The vomeronasal organ: roles in deciphering social cues and behaviors

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200 years after the discovery of the vomeronasal organ (VNO) by Ludvig Jacobson in 1813, intense research efforts

are still ongoing to decipher the precise roles of the VNO in the detection of social cues and the control of instinctive and learned behaviors. The VNO of the mouse has emerged as the best-studied VNO model system and facilitates multidisciplinary approaches that involve a wide range of sophisticated techniques at the molecular, cellular, and whole animal levels. This lecture will discuss recent key findings from our group that have provided new insight into the identification of ligand families underlying vomeronasal recognition, the characterization of the role of specific molecules of the signal transduction machinery in vomeronasal sensory neurons (VSNs), and the identification of behavioral consequences caused by the activation of genetically defined VSN subpopulations. Together, these results advance our understanding of the mammalian VNO. Funded by Deutsche Forschungsgemeinschaft grants Schwerpunktprogramm 1392 and Sonderforschungsbereich 894.

## #214 Linking VNO function to the hypothalamus: GnRHR neuron spiking depends on female cyclicity

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Socially important chemosensory cues converge onto a small subset of hypothalamic neurons that produce gonadotropin-releasing hormone (GnRH), the master molecule of reproduction. Modulating reproductive physiology using a primary regulator, like GnRH, relies on the function of its downstream target cells expressing the GnRH receptor (GnRHR). Due to the scattered distribution and difficulty to identify these neurons, its role in neuronal excitability has not been elucidated. Using new genetic models, in which neurons in the brain can be unequivocally identified and targeted, we started to investigate the activity of GnRHR neurons to ultimately help us to examine the neural circuits involved in olfactory-encoded behaviors. Supported by the VolkswagenStiftung and by grants from the Deutsche Forschungsgemeinschaft (DFG SFB894 and SPP1392).

## #215 Sexual dimorphism and experience-dependent plasticity in vomeronasal sensory neurons

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In mice, the normal expression of most sex-specific behaviors requires an intact accessory olfactory system (AOS). AOS circuitry is known to be sexually dimorphic, at least in downstream areas such as the hypothalamus and medial amygdala.Here we asked whether the vomeronasal sensory neurons (VSNs) exhibit functional differences between the sexes. Using high-speed calcium imaging and a collection of sulfated steroids, we recorded over a quarter-million individual neurons from male and female mice. Of 17 observed functional types, most were present in equal abundance in males and females. However, two types were dimorphic, including an epitestosterone-selective type 100-fold more common in males than in females.To explore the mechanism generating this dimorphism, we examined the role of sensory experience, and discovered that male "noses" become indistinguishable from female noses after long-term exposure to the odors of female mice.This difference in VSN receptor type is by far the strongest sexual dimorphism ever reported in the mammalian central nervous system; that this dimorphism is determined entirely by experience indicates that a sensory system devoted to "innate" responses is strongly modulated by rearing conditions.

## #216 Emergence of novel chemosensors: from the immune to the olfactory system

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Formyl peptide receptor (FPR) genes are found in all mammals. They are expressed in immune cells and recognize disease and pathogen-related molecules. Recently, in some rodent species, gene duplication events followed by gene cluster invasion led to the intermingling of vomeronasal receptor and FPR genes. This accident correlates with a drastically different expression pattern of most FPRs in these species: their transcription is absent from immune cells, and is restricted to sensory neurons of the vomeronasal organ. Transgenic approaches suggest that acquisition of stable vomeronasal expression by FPR genes results from their hijacking of two separate cis-located signals: one that provides tissue-specific punctate transcription, and another that stabilizes this expression. This multilevel transcriptional control is likely not restricted to this evolutionary novelty, but may reflect a general process that regulates the expression of chemoreceptor gene repertoires.

## #217 Vomeronasal receptor families in the deer mouse Peromyscus maniculatus: Towards an evolutionary analysis

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Chemoreceptors are the first element in a complex pathway that can ultimately affect behavior -- changes in their biochemical properties or expression pattern have the potential

to modulate specific behavioral responses. In particular, the activation of sensory neurons in the VNO is directly associated with changes in behavior, such as aggression, avoidance or mating. To understand the diversity and evolution of pheromone systems in wild populations, we are studying deer mice (genus Peromyscus), which diverged from Mus approximately 25 MYA. Here, we report on the characterization of VNO receptors from the most widespread species, P. maniculatus. In total, we identified 150 and 90 putative V1R and V2R genes, respectively, in the Peromyscus genome, fewer than in M. musculus (239 and 120, respectively). While clades previously identified in Mus have representatives in Peromyscus, our phylogenetic reconstructions indicate that most gene duplications took place after the split between the two lineages; several clades show sign of lineage-specific expansion or contraction. These differences in the chemosensory receptor repertoires likely reflect the unique habitats as well as social and mating behavior of deer mice.

## #218 Pheromonal induction of social learning in mice

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Darcin is an involatile protein pheromone in male mouse urine that reliably stimulates female sexual attraction to spend time near male urine. Darcin also rapidly conditions preference for its remembered location such that females and competitor males prefer to spend time in the remembered site even when no scent is present. However, other cues interact to influence interest in a male's scent when it is present and thus influence contact with this pheromone. For example, males recognize their own individual scent and spend little time in contact with their own darcin, showing minimal remembered preference for their own urine location. On contact with darcin in a male's urine, females also learn an attraction to the individual odour of the male, an attraction remembered for a prolonged period. I will address some of the unique physical properties of darcin, the importance of the amount of pheromone produced by different males when signaling competitively and the effects of male urine signals on hippocampal neurogenesis. We have also started to address whether there are similar pheromones in other species that induce conditioned learning of the location of attractive scents.

## #219 Specialized odors and neurons that generate innate behavior

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Survival behaviors such as aggression, fear, and mating are highly conserved across evolution. Their proper regulation and display is essential for fitness and requires neural activity from the amygdala and hypothalamus. However, the identity of the precise neurons and circuits that generate these survival behaviors remains largely unknown and therefore unstudied. In the mouse, all of these essential behaviors can be robustly initiated by olfactory cues. We have identified specific bioactive ligands that now enable us to precisely stimulate and identify the neural mechanisms that generate behavior. We are creating and assessing novel tools to be able to identify and manipulate the circuits that generate behavior. In addition, we are studying how the sensory information elicits variable responses depending on state, gender, or the complexity of the environment.

## #220 RNA-editing alters the function of vomeronasal formyl peptide receptors

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Recently we cloned all mouse formyl peptide receptors (Fprs) from vomeronasal mRNA and genomic DNA for functional studies in HEK cells. We now found clear evidence that Fpr function is modulated by RNA-editing in the vomeronasal organ (VNO). We observed a number of sequence variations between the genomic and VNO sequences in 3 of 5 cloned VNO Fprs. These variations were confirmed by direct sequencing. Subsequent studies revealed a critical impact of VNO-specific structural variations on the function of Fpr-rs1. We could readily activate the VNO variant of Fpr-rs1 but the genomic variant did not respond to any of ~ 100 tested compounds, although both variants are expressed at the cell surface. Most observed variations had typical hallmarks for RNA editing by adenosine deaminases (ADARs). RT-PCR experiments for all 3 different mammalian ADAR subtypes detected ADAR1 expression in the VNO. Immunostainings in OMP-GFP mice showed ADAR1 expression in most mature sensory neurons (VSNs). Given that Fprs are only expressed in ~4% of all VSNs it is likely that other genes are subjected to RNA editing. To our knowledge, this is the first report that RNA-editing leads to functional alterations in the olfactory system. Supported by SPP 1392.

## #221 Encoding strain and reproductive state by AOB neurons

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Adaptive social behavior towards conspecifics requires identification of both permanent (e.g. sex, genetic background) and transient characteristics such as the current reproductive status. In this study, we examined the neuronal

representation of the female mouse genetic identity and reproductive state by neurons in the first brain relay of the vomeronasal system, the accessory olfactory bulb. To identify which bodily secretions provide information to AOB neurons about these aspects, we tested responses in male mice to urine, saliva, and vaginal secretions from estrus and non-estrus BalbC and C57 female mice. We find that all secretions elicit responses in AOB neurons, and that while these responses can convey information about both genetic background and the reproductive state, genetic background is generally the more robustly encoded parameter. Furthermore, the response profiles of most individual AOB neurons are not consistent with a high level representation of either strain or state, suggesting that brain regions downstream of the AOB must integrate information from multiple channels before extracting behaviorally relevant information.

## #222 Olfactory control of behavior

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Chemical communication in mice controls complex social and sexual behaviors. Information about sex, age, social status, and individuality is conveyed by elusive chemical cues that are detected and processed by the olfactory system. These include sex pheromones that induce mating or aggression, predator odors that cause fear, and neonatal odors that elicit parental care. Furthermore, several mouse behaviors require an intact olfactory system but involve pheromones that remain elusive. We developed a high throughput strategy for pheromone identification, and identified several odors that evoke instinctive responses, including an aversive carnivore odor and an attractive, sexually dimorphic mouse odor. We also identified the receptors that mediate odor aversion and attraction responses, providing a foundation for dissecting underlying neural circuitry. A goal of our research is to characterize ligands, receptors, and neural circuits that mediate odor-driven behaviors.

## #301 Finding the right stuff - olfactory-based resource location in Drosophila

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Drosophila flies are heavily dependent on the sense of smell to locate optimal feeding and oviposition sites. We dissect neural circuits involved in these contexts. Specific assemblies of olfactory sensory neurons on antenna and palp detect olfactory information. Some neurons are specifically involved in egg laying, others in detecting optimal food sources. Interestingly, detection of cues involved in attraction and oviposition behavior, respectively, seem to be uncoupled. Here I will present very recent data describing both peripheral and central events directly underlying behavioral sequences strongly correlated with fitness.

## #302 In hawkmoths pheromone transduction is not primarily based upon an Orco-dependent ionotropic mechanism but on a phospholipase C-dependent metabotropic cascade

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Function of the conserved coreceptor (Orco) in insect odor transduction is still under debate. In primary cell cultures of olfactory receptor neurons (ORNs) of the hawkmoth Manduca sexta pheromone elicits a sequence of ion channel activations which can also be mimicked via perfusion with inositol trisphosphate (IP3). Thus, we challenged the hypothesis that moth pheromone transduction solely involves phospholipase C (PLC)-dependent metabotropic cascades. With ELISAs daytime-dependent changes in IP3 levels in hawkmoth antennae were shown with a maximum during the activity phase. In addition, pheromone-dependent increases in IP3 levels occurred in the presence of protein kinase antagonists and PLC agonists. In tip recordings of pheromone-sensitive sensilla in vivo PLC antagonists decreased pheromone responses while Orco agonist VUAA1 had no effects. However, VUAA1 activated spontaneous activity dose-dependently. Thus, while there is no evidence for Orco-dependent ionotropic pheromone transduction in hawkmoths, evidence accumulates for G-protein-dependent PLC activation as the primary mechanism of pheromone transduction. [Supported by DFG SPP 1392, STE531/20-1,2]

## #303 Sensitivity regulation in insect odorant receptors

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Insects odorant receptors (ORs) are composed of an odorant-specific protein OrX and a ubiquitous coreceptor (Orco) and form ligand-gated ion channels. Orco proteins expressed alone form also channels that are activated by cAMP. Stimulation of cAMP production in insect olfactory sensory neurons enhances the odor response. Moreover, repeated sub-threshold odor stimulation sensitizes the ORs. The sensitization can be mimicked by Orco-activating signalling and prevented by inhibition of cAMP production. Furthermore, flies expressing Orco mutants insensitive to cAMP are not sensitized by sub-threshold odor stimuli. These results indicate that Orco-mediated regulation of OR sensitivity provides tunable ionotropic receptors. This allows OR-expressing neurons to detect odors over a wide range of concentrations, especially to respond fast and highly

sensitive to brief stimulation. Supported by the Max Planck Society and the DFG (SPP 1392).

## #304 Structure, function, and biology of the insect olfactory co-receptor Orco

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First identified in 1999, insect odorant receptors are now known to be heteromeric protein complexes composed of a variable ligand-selective subunit and a constant co-receptor subunit called Orco. Essentially nothing is known about the subunit stoichiometry or how and where odorants interact with the complex. In recent years, two hypotheses for the signaling mechanism have been proposed. In the first, the OR/Orco complex functions ionotropically as an odor-gated cation channel. In the second, this ionotropic mechanism is wholly or partially subsumed by a metabotropic mechanism. I will review experimental evidence from my group that addresses the structure and function of this family of receptors.

## #305 Hedgehog signaling regulates odorant receptor cilia localization in Drosophila

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Olfactory sensory neurons (OSNs) adapt to the odor levels in the environment by a crucial but poorly understood process. In general, receptor concentration on a cell membrane defines partly the strength of the cell response to the ligand and odorant receptors define the odor response for each OSN. Here, we demonstrate that Drosophila Hedgehog (Hh) signaling is required for odorant receptor (OR) transport to the OSN cilia. Cilia localization and function of odorant receptors in Drosophila requires a coreceptor, Orco. Surprisingly, Orco localize to cilia in absence of Hh. In addition, our results clearly show that Hh signaling is not required for cilia transport in general, which implies that Hh specifically regulate OR cilia localization and function. Our results further demonstrate that OSN activity regulates Hh expression and reveal an autoregulatory loop that can define the concentration of OR on the cilia membrane.

## #306 Understanding plasticity in the olfactory intrabulbar map

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In mammalian olfactory system sensory neurons project their axons to the surface in the olfactory bulb generating a pair of glomerular maps that reflect odorant receptor identity. These maps are further connected through a set of reciprocal intrabulbar projections that are mediated by tufted cells that specifically link iso-functional odor columns to produce a second order map called the intrabulbar map. We have shown that intrabulbar projections are established postnatally and undergo continuously refinement through an activity dependent process that has no critical period. Our work seeks to understand the basis of this ongoing network plasticity as we explore the relationship between sensory-induced activity, neural regeneration and circuit remodeling in the olfactory bulb.

## #307 Local neuropeptide signaling promotes the integration of newborn neurons in the olfactory bulb

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Through ongoing neurogenesis, the mammalian olfactory system exhibits a remarkable level of activity-dependent circuit plasticity. To reveal mechanisms that link neural activity to continued circuit formation, we sought to identify cell types that provide presynaptic inputs onto adult-born neurons. By transsynaptic tracing, we identified local corticotropin-releasing hormone (CRH)-expressing neurons as providing extensive presynaptic input onto newborn neurons. We found that adult-born neurons dynamically express the CRH receptor during critical periods of synaptogenesis, and that CRH signaling is required for normal circuit integration. Additionally, gain of CRH receptor function in adult-born neurons leads to enhanced synaptogenesis in vivo. We have previously identified reciprocal connectivity between mitral cells and CRH-expressing neurons, suggesting a cellular link between olfactory stimulation, CRH signaling, and continued circuit integration of adult-born neurons. Together, these data reveal a novel mechanism by which neuropeptide signaling promotes synapse formation and cell survival, linking neural activity to downstream signaling pathways that underlie continued neural circuit plasticity in the adult brain.

## #308 Inhibition and plasticity in the mouse accessory olfactory bulb

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The mammalian accessory olfactory bulb (AOB) guides innate behaviors by encoding chemical cues important for gender discrimination and individual recognition. The AOB is also an attractive model for sensory learning, expressing robust changes in pheromonal processing after mating. Both normal processing and plasticity appear to be strongly regulated by local inhibitory circuits in AOB, but the properties of synaptic inhibition are poorly understood. Here we use in

vitro electrophysiology and imaging to test how inhibitory feedback shapes output from AOB, and how inhibition is affected after mating. In naive animals, spike trains in AOB mitral cells drive slow inhibitory feedback that accumulates over timescales of many seconds, matching the slow time course of natural sensory responses. Once recruited, feedback inhibition strongly reduces mitral cell output. In slices from recently mated animals, both the speed and degree of late inhibitory suppression appear to be increased. The slow temporal profile of inhibition may filter sensory input by preserving initial phasic components that guide rapid behavioral responses, but reducing extended bouts of firing that could drive slower changes in hormonal state.

## #309 Independent control of gamma and theta activity by distinct interneuron networks in the olfactory bulb

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Circuits in the brain possess a remarkable ability to orchestrate activities on different timescales, but how circuits interact to sculpt diverse rhythms remains unresolved. The olfactory bulb (OB) is a classic example where slow, theta, and fast, gamma, rhythms coexist. Moreover, inhibitory interneurons, which are often implicated in rhythm generation, are segregated into distinct layers, neatly separating the local, feed-forward circuit motifs from global, lateral components. Here, we combined intracellular recordings in vivo from identified inter- and projection neurons with circuit-specific optogenetic interference to dissect the contribution of inhibition to rhythmic activity. We found that the two inhibitory circuits control rhythms on distinct timescales: The local, glomerular network coordinates slow, theta activity, regulating baseline rhythms and odour-evoked inhibition. Granule cells on the other hand orchestrate gamma synchrony and spike timing. Surprisingly, they do not contribute to theta activity and odour-evoked inhibition despite their perceived dominance in olfactory bulb function. This suggests dissociable control of activities on distinct time scales by separate inhibitory networks within the olfactory bulb.

## #310 Electroencephalographic responses to chemosensory stimuli

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Until now, the electroencephalographic (EEG) responses to chemosensory stimulation have been identified mainly using across-trial averaging in the time-domain. This procedure cancels out changes in the EEG signal that are not strictly time-locked and phase-locked to the stimulus onset and, thereby, enhances the signal-to-noise ratio of time-locked ERP. Using such an approach the EEG responses to chemosensory stimulation have been characterized as a negative wave peaking approximately 320–500 ms after stimulus onset (N1), followed by a late positive wave peaking approximately 450–800 ms after stimulus onset (termed as P2 and/or P3). Conventional time-domain averaging presents some drawbacks, which could contribute to the low signal-to-noise ratio of chemosensory ERPs; temporal jitter, impossible to reveal any transient event-related modulation of the power of ongoing EEG oscillations. Taken together, time-domain averaging is thus blind to a significant fraction of the elicited EEG responses. This could contribute to explain why CSERPs are sometimes difficult to identify even in healthy subjects.

## #311 Olfactory and trigeminal event-related potentials compared in a spatiotemporal domain

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In this study we aimed to compare the human olfactory and trigeminal processing in a spatiotemporal domain. A trigeminal and a olfactory stimulus (CO2 and H2S), were delivered with an olfactometer to young, healthy volunteers, while the high density electrodes (64) EEG was recorded. The analysis was performed in a classical (5-electrode, main ERPs peaks) and modern approach (high topographical resolution, inverse solution, source localization). Results of microstates segmentation highlighted 5 maps that generally described the two processes at cerebral level. The trigeminal response differed from the olfactory response up to 300ms after stimulus onset. In this time range, source analysis pointed out that the olfactory stimulation involved olfactory related areas, while trigeminal stimulation involved noxious/somatosensory specific brain areas. Moreover, from 300ms on our data showed a similarity between the two processes. Statistical parametrical mapping of the differences between conditions suggested greater activation in a specific motor/sniffing network for the CO2 stimulation (probably related to a regulation of the potential noxious stimulus) and a greater activation of the ipsilateral primary olfactory cortex for H2S.

## #312 Genome and transcriptome analyses of human olfaction

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Our goal is to understand molecular genetic aspects of human odorant sensitivity, including specific as well as general anosmia, the latter including congenital general anosmia (CGA). For the former, we performed in depth analyses of next-generation RNA sequencing results of human and mouse olfactory epithelium and mouse olfactory bulb, focusing both on olfactory receptor (OR) and olfactory auxiliary (OA) genes (cf. Keydar et al, Human Mutation 2013). We noted inter-paralog differential OR expression, as well as novel aspects of OR gene structure. For OA genes, our analyses help clarify which paralogs may have an olfactory function. An example is an olfactory epithelium-enriched cytochrome P450 2G2, currently being investigated for a role in odorant processing, with implications to specific anosmia. For some OA genes we see evidence for olfactory-related splice variants, manifested in potential new olfactory specific exons. In parallel, we applied whole-exome sequencing to 20 individuals in 8 CGA families, in search for causative genes. In one family we identified a relevant rare missense variation in the transcription regulator ZXH1 gene, suggested via its interacting proteins to play a role in olfactory bulb development.

## #313 Deorphanization and characterization of human odorant receptors in heterologous cells

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Olfaction plays an indispensable role in human and animals in self and environmental recognition as well as intra- and inter-specific communication. Following the discovery by Buck and Axel in 1991 of a family of odorant receptors (OR), it has been established that the sense of smell begins with the molecular recognition of a chemical odorant by one or more ORs expressed in the olfactory sensory neurons. Therefore characterization of the molecular interactions between odorant molecules and ORs is a key step in the elucidation of the general properties of the olfactory system and in the development of applications: design of new odorants, search for blockers,… The presentation will show the process put in place at ChemCom to deorphanize and to characterize the interaction between chemical odorants and ORs. The family of human ORS includes ~400 putatively functional ORs which are GPCRs. To date over 100 hORs have been deorphanized.

## #314 Evidence for a genetic basis of olfactory loss in humans on chromosome 1

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Olfactory function varies among individuals. Factors that explain this variability are poorly understood. We sought to examine the genetic basis of variation in olfactory function. We used the Brief Smell Identification Test to assess odor identification of 659 adults with normalized environmental influences due to communal living. We used the Genome-wide Efficient Mixed Model Association algorithm to test for association between genotype and olfactory function to identify SNPs underlying susceptibility to decreased olfaction, adjusting for age and population structure. Using gene-based methods, a signal within the olfactory receptor (OR) gene cluster on chromosome 1 was identified. One strong candidate in this region is OR2M7, a gene that accounted for the second strongest evidence for association in the genome ($P<2.2E-04$). Furthermore, the SNP with lowest p-value in this region is in near perfect linkage disequilibrium with a non-synonymous SNP 65 Kb away within OR2M7 which has previously associated with asparagus anosmia.Our data support the role of genetic variation in susceptibility to olfactory loss in humans. More data are needed to understand the contribution of genetic factors to olfactory function.Funding: NIA

## #315 Non-synaptic inhibition between grouped neurons in an olfactory circuit

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Diverse sensory organs, including mammalian taste buds and insect chemosensory sensilla, show a striking compartmentalization of receptor cells. However, the functional impact of this organization remains unclear. Here we show that compartmentalized Drosophila olfactory receptor neurons (ORNs) communicate with each other directly. The sustained response of one ORN is inhibited by the transient activation of a neighboring ORN. Mechanistically, such lateral inhibition does not depend on synapses and is likely mediated by ephaptic coupling. Moreover, lateral inhibition in the periphery can modulate olfactory behavior. Together, the results show that integration of olfactory information can occur via lateral interactions between ORNs. Inhibition of a sustained response by a transient response may provide a means of encoding salience. Finally, a CO2-sensitive ORN in the malaria mosquito Anopheles can also be inhibited by excitation of an adjacent ORN, suggesting a broad occurrence of lateral inhibition in insects and possible applications in insect control.

## #316 The on and off of mammalian olfactory transduction

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Sensory transduction is a cellular process by which receptor cells convert sensory stimulation into changes in membrane potential. In vertebrates, primary olfactory sensory neurons use a G protein-coupled, cyclic AMP second messenger-mediated signaling pathway to transduce odors. Binding of the odorant to its receptor leads to membrane depolarization, which eventually triggers action potential. Research over a quarter of a century has recognized that seven protein or protein complexes constitute the major components of the mammalian olfactory transduction pathway, with five responsible for activation and two for termination. Identification of these components is a necessary step towards understanding how humans and animals smell. Recent studies using a proteomic approach contributed to elucidating the molecular identity of two transduction components. Studies using a molecular genetic approach also suggested that calcium extrusion via calcium transporter, rather than the degradation of cyclic AMP, governs the rate of signal shutoff.

## #317 Sensing danger cues via the olfactory Grueneberg ganglion

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In mammals, the most recently discovered olfactory subsystem, the so-called Grueneberg ganglion (GG), is present at the tip of the nose, close to the opening of the naris. This organ was first described by Hans Grueneberg in 1973, as a 'ganglion' of unknown function. The GG is an arrow-shaped neuronal structure at the anterior end of the nasal cavity that lines both sides of the nasal septum. In an adult mouse, from 300 to 500 cells can be found in each GG. This organ has been proposed to mediate alarm pheromone (AP) detection in mice. Recently, we have been able to identify the precise chemical structure of one mouse AP, the SBT (2-sec-butyl-thiazoline). Since then, we have also identified several new ligands activating the mouse GG neurons. They are very closely related to the identified mouse AP. They share similar chemical features as the sulfur-containing volatiles that are released by predating carnivores. Sensing these chemical warnings present in the environment is essential for species survival. These findings thus not only reveal a chemical Leitmotiv that underlies signaling of fear, but also point to a double role for the olfactory Grueneberg ganglion in intraspecies as well as interspecies communication of danger.

## #P001 Vomeronasal sensory neurons show both determined and variable stimulus coding strategies

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In contrast to combinatorial odor coding by neurons in the main olfactory epithelium, vomeronasal sensory neurons (VSNs) are thought to function as narrowly tuned, dedicated sensors of intrinsically instructive semiochemicals. Here, we investigate the tuning profile(s) of a group of VSNs that are characterized by their sensitivity to a specific class of chemosignals - major urinary proteins (MUPs). Using extracellular patch-clamp recordings from basal VSNs in acute coronal VNO slices, we recorded stimulus-dependent action potential discharge in response to recombinant MUPs. Furthermore, we comparatively analyzed the role(s) of these stimuli in two different male-specific behaviors: aggression and territorial countermarking. Surprisingly, electrophysiological activity profiling revealed parallel detection of MUPs by both 'specialist' neurons selectively tuned to a particular stimulus and broad range responders ('generalists') sensitive to all or subset combinations of the MUPs tested. These data suggest the coexistence of determined and variable sensory coding strategies in the mouse VNO. In addition, behavioral assays indicate that dedicated ligands promote aggression, whereas a combinatorial MUP code controls countermarking.

## #P002 Clinical analysis of 1284 patients with taste disorders

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Taste disorders are caused by several factors, and there have been few reports concerning the clinical course of taste disorders. In this study, patients with taste disorders were classified into 11 groups according to causes, and they were retrospectively studied in terms of therapeutic effects. In total, 1284 patients (509 men and 775 women, mean age: 60.6 years) who complained of taste disorders were reviewed in our clinic.The causes were idiopathic taste disorder (233 cases, 18.1%), psychogenic (229 cases, 17.8%), drug-induced (227 cases, 17.7%), post-common cold (174 cases, 13.6%), zinc deficiency (149 cases, 11.6%), systemic disease (101 cases, 7.9%), iron deficiency (44 cases,

3.4%), iatrogenesis (68 cases, 5.3%), traumatic (41 cases, 3.2%), central nervous system disease (4 cases, 0.3%), and the others (14 cases, 1.1%).The drug-induced taste disorders were the longest recovery period in the other groups. In the patients who were able to start treatment within 6 months from the onset of taste disorder, the recovery rate was significantly higher ($p<0.05$) and the therapeutic period was significantly shorter ($p<0.0001$) than in those who had the disorders for more than 6 months.

## #P003 Possible roles of Robo1-positive ensheathing cells in guiding dorsal-zone olfactory sensory neurons in mouse

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In the mouse olfactory system, the anatomical locations of olfactory sensory neurons (OSNs) correlate with their axonal projection sites along the dorsoventral axis of the olfactory bulb (OB). We have previously reported that Neuropilin-2 expressed by ventral-zone OSNs contributes to the segregation of dorsal and ventral OSN axons, and that Slit is acting as a negative land mark to restrict the projection of Robo2+, early-arriving OSN axons to the embryonic OB. Here, we report that another guidance receptor, Robo1, also plays an important role in guiding OSN axons. Knockout mice for Robo1 demonstrated defects in targeting of OSN axons to the OB. Although Robo1 is co-localized with dorsal-zone OSN axons, it is not produced by OSNs, but instead by olfactory ensheathing cells. These findings indicate a novel strategy of axon guidance in the mouse olfactory system during development.

## #P004 Functional effect of a SNP of TAS2R16 region associated to human longevity

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We have recently shown that a single nucleotide polymorphism (SNP), rs 978739 (A/A), which maps 212 b.p. upstream the coding region of the TAS2R16 gene, is associated with an extension of longevity in a population of elderly. We have cloned the promoter region of the gene TAS2R16, including rs 978739, (A/A) in the vector PGL3, upstram the luciferase gene. Two cell lines, SAS, and HCT116, were co-transfected either with the plasmid bearing PGL3 alternatively the two allelic variants (A/A and G/G), and with the PRL-TK vector carrying the gene of renilla.In both cell lines it was observed a increased basal expression of the luciferase gene when the variant A/A was present, compared to the variant G/G. SAS transfected cells were also treated with salicin which is a effective ligand of the receptor encoded by the gene TAS2R16. Treatments with salicin 0.2 mM for 10 hours induced a greater expression of luciferase, suggesting that the salicin is able to induce the expression of its own receptor. In conclusion we have shown a functional role of this SNP, in the region upstream of the gene coding for the TAS2R16 receptor, that is associated with a greater extension of human longevity.

## #P005 Temporal analysis of AOB response profiles

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One of the characteristics of the vomeronasal system is its slow time course which is probably determined by the dynamics of vomeronasal sensory neurons, the vomeronasal pump and by early stages of neuronal processing within the accessory olfactory bulb. To investigate whether the temporal dynamics of vomeronasal responses convey information about sensory stimuli, we examined the reliability by which responses to various secretions at several concentrations can be distinguished. Our preliminary analyses indicate that the optimal temporal window for discrimination depends on the neuron in question and on the specific cues which must be discriminated. These results thus suggest that despite the slow time course of vomeronasal responses, downstream processing stages can obtain more information from AOB neuronal responses by taking the dynamic temporal response profiles into account.

## #P006 Fruit flies like a banana: Graded encoding of food odor value in the Drosophila brain

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Odors are highly evocative, yet how and where in the brain odors derive meaning remains unknown. Our analysis of the Drosophila brain extends the role of a small number of hunger-sensing neurons to include food-odor value representation. In vivo two-photon calcium imaging shows the amplitude of food odor-evoked activity in neurons expressing Drosophila Neuropeptide F (dNPF), the Neuropeptide Y homolog, strongly correlates with food-odor attractiveness. Hunger elevates neural and behavioral responses to food odors only, though heightened responses also exist for some food odors when fed. Inactivation of a subset of dNPF-expressing neurons or silencing dNPF receptors abolishes food-odor attractiveness, whereas genetically-enhanced

dNPF activity not only increases food-odor attractiveness but promotes attraction to aversive odors. Varying the amount of presented odor produces matching graded neural and behavioral curves which can function to predict preference between odors. We thus demonstrate a possible motivationally-scaled neural 'value signal' accessible from uniquely identifiable cells.

## #P007 Olfactory imprinting in zebrafish: Combinatorial analysis of Ca-binding proteins in olfactory sensory neurons and their primary projections

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We present here a combinatorial analysis of four Ca-binding proteins (CBPs) including their expression in olfactory sensory neurons (OSNs) as well as their axonal projections to the olfactory bulb (OB). We confirm a major expression of calretinin and S100 in ciliated and crypt cells, respectively, with some expression of S100 in microvillous cells. Parvalbumin is strongly expressed in ciliated and microvillous cells. Calbindin is present in ciliated and microvillous cells, but restricted to ciliated cells in adults. Microvillous cells are divided at least in a parvalbumin+ and a parvalbumin+/S100+ subpopulation. OB projections visualized with CBPs show subtle differences, e.g. S100+ terminals are only seen in a mediodorsal glomerulus and develop after day 3. Parvalbumin+ projections into this and other glomeruli appear much earlier. Possibly, these S100 fibers develop in correlation with the olfactory imprinting process (day 6). Also, a ventromedial glomerulus receives parvalbumin, but not calretinin fibers. In conclusion, CBPs combinatorially define subpopulations of OSNs. It will be interesting to see if for example the two subpopulations of microvillous cells correspond to V1R and V2R receptor cell populations.

## #P008 New insights on the role of the CNGA4 protein in the mouse vomeronasal organ

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Mammalian pheromones are key chemical signals in the regulation of social behaviors and are recognized by the vomeronasal organ (VNO). Pheromone detection takes place on the dendritic microvilli of VNO sensory neurons (VSNs). In 2001, the TRPC2 channel has been demonstrated to be one of the key effector of the pheromone signaling pathway. Nevertheless many other ion channels are expressed on microvilli of VSNs such as the CNGA4 protein. Its precise role in the pheromone signaling pathway remains mostly unknown. We therefore decided to study its role in the VNO. We observed the protein to be expressed on VSNs microvilli and to indeed play a role in the pheromone signaling pathway as mice lacking the CNGA4 protein display a modified social behavior. In parallel, we observed with in vitro experiments using HEK cells that the CNGA4 protein could directly interact with TRPC2 acting either as a chaperon or as a subunit of a heteromeric channel. These results give us some new insights on the combined roles of vomeronasal transduction channels.

## #P009 Evolution of formyl peptide receptor function in mammals

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We recently used high throughput calcium imaging to compare the function of formyl peptide receptors (Fpr) of the immune and vomeronasal (VNO) system. We found a functional conservation between mouse and human immune Fprs and provided evidence for high specificity of at least one VNO Fpr. Moreover, we observed that the ligand profile of Fpr-rs1 is consistent with a role in VNO pathogen sensing. We now compared the functional response of human, mouse, rat, dog, and rabbit Fprs to more than 100 agonists. We identified several new ligands for immune Fprs. Structure-function studies suggest widespread conservation of a specific ligand recognition site and show that response profiles are highly conserved across species. Thus, evolutionary constraints of immune Fpr-function seem very similar across mammals, although each species faces species-specific pathogens. In contrast, we observed that VNO-Fprs responded to very few tested agonists. We identified two functional orthologs of mouse Fpr-rs1 that are both very narrowly tuned. We conclude that divergent evolutionary constraints cause distinct agonist properties of immune and VNO-Fprs. Sup.: SFB894, HOMFOR2011

## #P010 Naming odors takes (a lot of) time

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It is well known that it is difficult to label odors in the absence of contextual cues. Relatively less is known about the latency of naming odors and the consistency with which people can do such a task. The current study measures people's ability to name common and uncommon odors and, for comparison, pictures, as well as the speed and consistency of their responses. Method: Twenty-four college

students (12 male) produced verbal labels for 50 images and 50 odors. Half of the odors and pictures were common and half were uncommon. Sixty percent of the stimuli were shown twice. Results: Participants provided fewer labels for odors (<40%) than for pictures (100%). Common pictures were labeled nearly perfectly, consistently and very quickly (~1000 msec), but uncommon pictures and common and uncommon odors were not consistent (~50%, <40% and <20%, respectively) and were relatively slow (3000–6000 msec). Accuracy was <40% correct for odors, but even accurately labeled odors were named slowly. Discussion: The results extend our understanding of the difficulty of naming odors, particularly relative to the ease of naming pictures. These results suggest that odor naming is a laborious process and may serve little ecological value.

## #P011 Response of the tortricid pest Lobesia botrana to volatiles emitted by the non-host plant Perilla frutescens

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The European Grapevine Moth Lobesia botrana (Denis & Schiffermüller) is a major pest of grape worldwide. Non-host plant compounds which elicit the so called somatosensory sensation, have been used in agriculture for their ability to interfere with insects. Among these, Perilla frutescens (L.) compounds were shown activating sensory Transient Receptor Potential (TRP) channels, which are also involved in the perception of somatosensory-compounds, and are expressed in tortricid antennae. In search of volatiles emitted from P. frutescens with a potential application in pest control, we screened essential oil metabolites for biological activity on the olfactory system of L. botrana.Electrophysiologically active compounds released from different P. frutescens varieties were identified by GC-EAD. In a dual choice oviposition test, females showed a preference for odors released by a variety whose profile is dominated by S-(-)-Perillaldehyde over a host-plant odor bouquet. In Y-tube olfactometer assays, virgin males showed a significant enhancement at the beahavioral level, in the presence of odors released by a variety whose profile is dominated by Perillaketone and Isoegomaketone.

## #P012 Real-time two-photon imaging reveals rapid and continuous plasticity of sensory input to the mouse olfactory bulb in vivo

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Olfactory sensory neurons (OSNs) regenerate throughout life, but whether the synapses that they form persist for the lifespan of the neuron, or turn over, is not known. To address this question, we expressed tdTomato and synaptophysin-GFP (sypGFP), a presynaptic marker, specifically in either immature (Gγ8 promoter) or mature (olfactory marker protein promoter) OSNs and performed 2-photon imaging in vivo in juvenile and adult mice through cranial windows implanted over the olfactory bulb (OB). Turnover of sypGFP clusters in the glomerular layer was evident on a timescale of hours, and was 3-fold higher for immature than for mature axons over 3 hours ($P<0.001$). Both formation ($P=0.004$) and loss ($P=0.001$) of Gγ8-sypGFP clusters occurred at higher rates than for OMP-sypGFP clusters. However, there were no differences in turnover rate, formation or loss of either OMP-sypGFP or Gγ8-sypGFP clusters between juvenile and adult mice ($P>0.56$). Our findings provide evidence for rapid and continuous turnover of sensory inputs to the OB even in adult mice, providing an important substrate for lifelong plasticity. Moreover, our data suggest that neuron maturity, rather than mouse age, is the key determinant of OSN axon structural plasticity.

## #P013 HCN channels mediate proton-dependent signaling in the mouse vomeronasal organ

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The mouse vomeronasal organ (VNO) plays a critical role in chemosensory communication. However, many physiological mechanisms underlying vomeronasal signaling remain largely elusive.Here, we examine proton-mediated activity in the mouse VNO. We show that urinary pH depends on gender, sexual experience and estrus cycle stages. In particular, sexually experienced female mice display a generally more acidic urinary pH, which significantly decreases during estrus. Using whole-cell patch-clamp recordings, we show that vomeronasal sensory neurons (VSNs) are proton-sensitive. We describe that acidic solutions dose-dependently activate most VSNs with threshold activation in the pH range of urine. Surprisingly, our data suggest no substantial involvement of 'classical' proton-sensitive ion channels or TRPC2 channels in pH-dependent signaling. Instead, the pharmacological profile and biophysical properties of the proton-induced responses indicate a critical role of hyperpolarization-activated cyclic-nucleotide-gated channels in proton-mediated signaling of VSNs. Together, our findings indicate a critical role of vomeronasal proton-sensitivity in gain control of social chemosignaling. This work is supported by the ECRO 2013 travel grant.

## #P014 An electroolfactogram study of odor response patterns from the mouse olfactory epithelium with reference to receptor zones and odor sorptiveness

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Vertebrate olfactory sensory neuron (OSN) responses tend to be spatially heterogeneous but similar across subjects for a given odor. One proximate explanation for these stereotyped patterns comes from evidence that olfactory receptor (OR) genes are expressed in OSN populations that are spatially restricted to zones in the olfactory epithelium (OE). A long-standing functional explanation for such response patterns posits that they are the signature of a supplementary mechanism for quality coding based on the sorptiveness of odor molecules. Here mouse OE response patterns were studied using the electroolfactogram (EOG). In all, 290 mice were used to record from a set of standard locations across the OE utilizing 11 odors resulting in >4,400 recordings. Results confirmed a marked spatial heterogeneity in responses that varied with odor. However, no discontinuities were found in the odor specific patterns across the OE as might have been predicted by receptor zonal distribution. Nor was clear support found for the hypothesis that OE response patterns have been shaped by relative odor sorptiveness. It is proposed that receptor zones and spatial patterning may be epiphenomena of a contingent evolutionary process that created ORN types.

## #P015 Deorphaning odorant receptors using in vivo synaptopHluorin imaging in transgenic and gene-targeted mice

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The molecular basis for odor perception was elucidated in 1991 with the cloning of odorant receptors (ORs), the largest family of G-protein coupled receptors. Since that time, intensive effort has been expended to identify specific odorous ligands for odorant receptors in mice, rats and humans. Nevertheless, most of the ORs remain to be 'deorphaned'. ORs are notoriously hard to express in vitro and therefore ligands must be identified in an in vivo setting. We have measured odorant responses from specific glomeruli in living mice using synaptopHluorin, a green fluorescent protein derivative, which modulates its fluorescence in response to neuronal activity. Using this genetically encoded probe we have systematically mapped the distribution of a large set of malodors along to the dorsal domain of the olfactory bulb, a region that is amenable to optical imaging. Further characterization of ORs that respond to aversive stimuli will revolutionize our understanding of human perception of malodors. In addition we are using gene-targeted mice to assess odor function for known and unknown receptors in the dorsal region of the bulb.

## #P016 Fast real-time PCR based screening for common aneuploidies in mouse ES and iPS cells

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We report a fast, accurate and inexpensive screen for identifying common aneuploidies in genetically manipulated mouse embryonic stem cells (ESC) and induced pluripotent stem cells (iPSC) using quantitative real-time PCR (qPCR). ESCs/iPSCs with aneuploidy prevent germ line transmission of a mutation while the standard conventional cytogenetic karyotyping methods by core facilities are time-consuming and often bypassed because of the extensive costs involved. We have set up a straightforward strategy for identifying Trisomy 8, and chrY aneuploidies; the only two aneuploidies that were consistently identified in 141 karyotyped ESC clones. Screening against these two aneuploidies significantly increases the fraction of normal ESC clones. Our method is extremely sensitive and can detect as low as 10% aneuploidy among a large population of mouse ESCs and iPSCs. It greatly expedites the generation of mutant mice and provides a quick tool for assessing the aneuploidy percentages of any mouse ESC or iPSC line.

## #P017 Activation of tongue-expressed GPR40 and GPR120 by non caloric agonists is not sufficient to drive preference

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Triglycerides, the main components of dietary fat, are hydrolyzed in the mouth by a lingual lipase secreted from the von Ebner gland and the released free fatty acids are detected by the taste system. GPR40 and GPR120 are expressed in taste bud cells and respond to fatty acids in vitro. Knockout mice lacking either of those receptors have diminished taste nerve responses to and reduced preference for fatty acids. Here we investigated whether activation of those GPCRs is sufficient to elicit fat taste and preference. Five non-fatty acid agonists of GPR40 and two non-fatty acid agonists of GPR120 activated the glossopharyngeal nerve of wild-type (WT) mice and this response was largely diminished in knockout mice lacking the cognate receptor. Non fatty acid

agonists of GPR40 dissolved in water were detected in sip and spit tests by human subjects and elicited a taste similar to that of linoleic acid whereas two agonists of GPR120 in water were not perceived fattier than water alone. WT mice did not show preference for five agonists of GPR40, two agonists of GPR120 and mixtures of both agonists over water in two bottle preference tests. These data indicate that GPR40 mediated taste perception is not sufficient to generate preference.

## #P018 Sensors for volatile chemicals: Room temperature ammonia and humidity sensing

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There have been extensive studies on conducting polymers and their use as chemical sensors especially for detection of odours. Among them, polyaniline (PANI) stands out due to its higher environmental stability and lower price. PANI is known to be sensitive to ammonia because of the high affinity of protonated N atoms on doped-PANI backbone to NH3. However, due to its lack of solution processability it is difficult to fabricate thin films of PANI. We have developed a vapour-phase deposition polymerisation (VDP) technique to in situ polymerise a very thin Nafion-doped PANI sensing layer on the flexible substrate. The conductivity of the sensor is reduced on exposure to ammonia even in a humid environment. The sensor shows sensitive, rapid and reversible response to very low concentrations of ammonia vapour in the range of 250–1500 ppb at room temperature. Moreover, the sensor shows a reversible increase in conductivity upon increasing humidity. Hence, a low-cost and flexible sensor for ammonia and humidity detection at room temperature is realised using a VDP method which is compatible with common solution-deposition techniques. The sensor can be used in smart tags for monitoring of perishable goods during the transportation chain.

## #P019 FMRP and structural plasticity of new neurons in the olfactory bulb

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The Fragile X Mental Retardation Protein (FMRP) is the protein whose absence leads to the Fragile X Syndrome (FXS), the major cause of hereditary intellectual deficiency. We showed that FMRP regulates the morphogenesis of newborn neurons in the adult olfactory bulb (OB) and that FMR1 mutated neo-neurons are incapable of reducing the length of their dendritic tree upon olfactory deprivation. We have developed a learning paradigm in FMR1-/- mice. Control or mutated mice are subjected to an olfactory perceptual learning paradigm. Strikingly, mutant mice could not learn to discriminate the two odorants, suggesting that FMRP is necessary for this type of learning. Interestingly, conditional inducible knocked-out mice (Nestin::CreERT2 X FMR1flox/flox) could not learn the task either, suggesting that FMRP absence in neo-neurons is at the origin of the learning deficit.Morphological analysis of wild-type neurons integrating during the enrichment period of the learning paradigm shows that perceptual learning induces a lengthening of their dendritic tree as well as an increase of spine density. In contrast, mutated neurons do not display any dendritic lengthening nor an increase in spine density.

## #P020 Determinants of pheromone preference in a moth

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Pheromone communication has served as a prime model for understanding the coding of olfactory preference and its evolution. Determinants of olfactory preference are however still largely elusive. Here we present data on how preference for and evolution pheromone in moths is reflected in the neurophysiology and molecular biology of its pheromone detection system. The species, Ostrinia nubilalis has a simple binary blend. In natural populations two strains exist, which produce and prefer opposite ratios of a two pheromone component blend. In spite of the numerous studies on the topic, the factor(s) that determine preference have not been surfaced, although the peripheral detection system seems to make the difference. Using double in situ hybridization with all combinations of pheromone receptors we present a layout of the pheromone detection system,

the differences between the two strains, and how this correlates with preference.

## #P021 The perplexing roles of olfactory marker protein in olfactory transduction

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Olfactory marker protein (OMP) is a small cytosolic protein expressed in mature olfactory receptor neurons (ORNs) with unclear function. ORNs lacking OMP have an odor response that is greatly prolonged and it has been demonstrated that OMP is a key component in speeding up cAMP kinetics by a hitherto unknown mechanism. Further, it has been shown that different olfactory receptors (ORs) have different basal (constitutive) activity thus driving basal fluctuations of cAMP. The aim of our study is to understand how OMP interact with elements of the cAMP signaling inside the cilia of the ORNs. We began to investigate the mechanisms of OMP interactions in an OR specific manner: we generated two OMP KO lines that also expressed GFP with either the low basal activity mOR-EG OR or the higher basal activity M71 OR. We found that OMP functions in an OR dependent manner, seeming to alter basal cAMP levels to a greater extent in ORNs expressing a quiet OR (mOR-EG) compared to a noisier (M71) OR. In short, our data show that the action of OMP is indeed OR dependent. We are trying to understand the exact mechanism by which OMP regulates cAMP levels and kinetics in an OR dependent fashion.

## #P022 Convergence of FPR-rs3-expressing neurons in the mouse accessory olfactory bulb

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In the mouse, most members of the FPR receptor family are expressed by vomeronasal sensory neurons. The neural circuitry corresponding to this class of chemical sensors is unknown. Taking advantage of the presence of FPR-rs3 on both vomeronasal dendrites and axonal fibers, we visualized the distribution of sensory cells expressing this member of the FPR family, and their corresponding axonal projections in the olfactory bulb. We found a rostrocaudal gradient of receptor choice frequency in the vomeronasal sensory neuroepithelium, and observed a convergence of FPR-rs3 axons into multiple, linked and deeply located glomeruli. These were homogenously innervated, and spatially restricted to the basal portion of the rostral accessory olfactory bulb. This organization, reminiscent of the one that characterizes axonal projections of V1R-expressing neurons, supports a role played by these receptors in the perception of semiochemicals.

## #P023 The contingent negative variation, a reliable marker of the activation of the olfactory system

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To check the transmission of the olfactory information to the brain, we used a paradigm to record, in addition to the usual OERP components, the contingent negative variation (CNV) induced by the perception of the odorant. The protocol allowed an estimate of the ability to detect the odorant by measuring the signal detection theory d' index from hits and false alarms rates during EEG recording. Two groups of subjects were investigated: 24 normosmic subjects and 8 patients deprived of both olfactory bulbs as shown by MRI 1.5T. The positive component P3 and the CNV were consistently observed in normosmic subjects, although individual variations were noted concerning the time course and amplitude of these signals. The presence of the CNV was particularly useful when the amplitude of P3 was small and difficult to distinguish from the background noise. Statistical analysis depicted a noticeable effect of age upon the onset and amplitude of P3 and the amplitude of P3 was negatively correlated to its onset. The index d' was $3.2 \pm 0.9$ for normosmic subjects and 0 for anosmic patients. In conclusion, the odorant-elicited CNV is a good complement to other OERP signals, and is a direct indicator of the perception of the odorant.

## #P024 Head trauma and olfactory loss

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We examined the olfactory dysfunction consecutive to a head trauma. The location and extension of the brain lesions were established by MRI, with a particular focus on the region of the olfactory bulbs. On each side, the transmission of the olfactory information to the brain was checked by OERP and CNV recordings, the ability to discriminate vanilla and phenethyl alcohol, and the signal detection theory d' index. The majority of traumas were consecutive to an occipital impact.

In most cases a lesion in the anterior part of the gyrus rectus was associated with a change in the appearance of the olfactory bulb located just below, which had an inhomogeneous aspect or a even was not visible due to ischemia. The OERP recording indicated either a lack of function and anosmia or, in some cases, a mono-lateral or bilateral residual activity of the olfactory pathway, associated with hyposmia and parosmia. In some patients the lesions were more extensive, particularly at the level of the frontal lobe and, sometimes also concerned the piriform cortex. This study suggests a coup-countercoup effect following the occipital impact and a partial or complete deterioration of the bulbs by local edema and compression.

## #P025 Recombinant Anoctamins form heteromultimeric complexes

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Recently, Anoctamin2 (Ano2) was identified as the molecular correlate of calcium-activated chloride currents in activated olfactory sensory neurons. However, the exact physiological role of Ano2 remains controversial. Here, we investigate expression and subcellular distribution of different Anoctamin family members in the mouse olfactory epithelium. Using deep sequencing and super-resolution microscopy, we find robust expression of Ano1, 2 and 6 and analyze their ciliary localization. Moreover, we analyze potential multimeric interaction(s) and functional properties of recombinant Ano1, 2 and 6 in heterologous cells. First, patch-clamp and calcium imaging recordings confirm the function of Ano1 and Ano2 as calcium-dependent chloride channels (CaCCs). Furthermore, Anoctamin6 also forms a CaCC, albeit generating considerably smaller currents. Next, using bioluminescence resonance energy transfer (BRET), we investigate potential heteromerization of different Anoctamin combinations. Our results suggest that recombinant Anoctamins interact to form heteromultimeric complexes. On-going experiments aim to characterize the functional effect of these interactions.

## #P026 In vivo characterization of an antennal carboxylesterase involved in pheromone response in Drosophila

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Insects respond to the spatial and temporal dynamics of a pheromone plume, which implies not only a strong response to 'odor on', but also to 'odor off'. This requires mechanisms geared toward a fast signal termination and pheromone-degrading enzymes (PDE) could be good candidates to rapidly inactivate odorants in the vicinity of the sensory receptors. Here we verified the role of a putative Drosophila melanogaster PDE, the carboxylesterase Est-6, on the response to the pheromone cis-vaccenyl acetate (cVA). We found that Est-6 is highly expressed in cVA-sensitive sensilla and that this enzyme strongly influences the dynamics of olfactory receptor neuron (ORN) responses, as in Est-6° null mutants cVA-sensitive ORN showed increased firing rate and prolonged activity. Mutant males had a lower threshold of behavioral response to cVA, in particular, mutant males exhibited a strong decrease of male-male courtship, in association with a delay in courtship initiation. Our study presents evidences that this enzyme plays a role in the physiological and behavioral dynamics of sex pheromone response in Drosophila as a putative PDE in male antennae.

## #P027 Chemo- and thermosensory signaling in the Grueneberg ganglion

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The Grueneberg ganglion (GG) - a cluster of neurons in the anterior nasal region – is considered as an olfactory organ. We have recently identified odorants activating GG neurons. Responsiveness to these odorants occurred in GG neurons characterized by the expression of the olfactory receptor V2r83, the guanylyl cyclase GC-G and the cyclic nucleotide-gated ion channel CNGA3. Experiments with knockout animals disclosed that GC-G and CNGA3 are important for odor-evoked GG responses.GG neurons were also found to be activated by cool temperatures. Investigating the relevant signaling mechanisms revealed that almost all V2r83-/GC-G-/CNGA3-positive GG neurons responded to coolness, i.e, the same subset of GG neurons is activated by coolness and the above mentioned odorants. Experiments with GC-G- and CNGA3-deficient mice showed that these elements contribute to coolness-evoked GG responses. Searching for a thermosensor, expression of the thermosensitive ion channel TREK-1 was observed in numerous GG neurons. Moreover, in TREK-1-deficient mice, GG responsiveness to coolness was reduced. However, even in the absence of TREK-1, GG neurons responded to cool temperatures, suggesting that TREK-1 is not the only thermosensor in the GG.

## #P028 Birth and migration of zebrafish olfactory sensory neurons

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A common feature of olfactory receptor (OR) gene expression, which is conserved across phyla, is the zonal organization of olfactory sensory neurons (OSNs) expressing the same OR. In zebrafish, OR expression domains form concentric patterns with OR-specific diameters. The functional significance of zonal OR expression is not well understood, but it is often related to the mechanism by which OSNs selects a single OR gene for expression: spatial restriction would simplify the selection process by limiting the OR repertoire from which a given OSN can choose. Here we provide evidence, that the apparent zonal organization of OR expression patterns in zebrafish is established secondarily and after OSNs transcribe OR genes. In adult olfactory tissue, OSNs are continuously replaced from two distinct proliferation zones located at each end of the sensory epithelium. OSNs adopt OR identity shortly after they exit mitosis and actively migrate across the olfactory tissue. Along their migratory route they sort out by OR identity and establish a zonal pattern of OR expression. Our findings thus challenge the concept that zonal OR expression patterns emerge from a spatially restricted OR gene choice mechanism in zebrafish.

## #P029 Mining the zebrafish olfactory transcriptome

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The molecular mechanisms that control olfactory receptor (OR) gene expression remain elusive. Current models invoke long-range control by distant enhancer sequences as well as short-range regulation by proximal promoter elements. Here we use transcriptome analysis in zebrafish to study expression of the complete OR gene repertoire. We find that a total of 166 previously known and 13 novel OR genes are expressed in the zebrafish olfactory epithelium. By mapping OR transcripts to the current zebrafish genome we identify their transcription start sites (TSSs), their intron-exon boundaries, and their relative levels of expression. About 75% of OR transcripts contain spliced 5'-introns, while no splicing events were detected in 3'-untranslated sequences. Analysis of OR expression highlights a curious modulation of transcript levels along OR gene cluster and across chromosomes pointing at candidate long-range regulatory sites that may control OR expression at the level of OR gene clusters. Using unsupervised bioinformatic analysis tools, we identified conserved sequence motifs proximal to the TSSs of OR genes, which provide candidate short-range regulatory sites that may control specific properties of OR expression.

## #P030 Taste processing in congenital anosmics: an fMRI study

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The tongue and nose contribute equally to the appreciation of food flavors. Without a functional sense of smell, anosmic individuals can only rely on taste and trigeminal information for identifying flavors. We examined the neural correlates of taste processing in a group of 10 Faroese family members affected by an isolated form of congenital anosmia (CA). We also included 9 normosmic controls (NC). Using event-related fMRI, participants had to identify 3 tastants (sweet, salty, bitter) that were delivered to the mouth using a gustometer. To test for group differences in taste processing, we performed a second-level analysis at the group level using the contrast "ALL TASTES vs. SOLVENT". In both groups, tasting activated the right insula, bilateral parietal opercula and pre/postcentral gyri together with the medial orbitofrontal cortex (OFC). Interestingly, NC participants recruited more strongly than CA participants the OFC and parietal operculum. Our data are the first to demonstrate that the loss of a chemical sense from birth does not lead to a functional reorganization of the brain. This highly contrasts with congenital blindness or deafness where the deprived cortex is involved in a variety of sensory and cognitive tasks.

## #P031 Delayed attenuation of taste neophobia induced by perirhinal cortex lesions and aging in rats

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Aging does not impair taste neophobia although it induces a slower attenuation of taste neophobia than that seen in adult rats since they need a higher number of taste preexposures for the taste to become safe (Morón and Gallo, 2007). The effect cannot be attributed to age differences in

taste sensitivity and it has been proposed to depend on age-related changes in the brain mechanisms relevant for detecting taste familiarity (Gámiz and Gallo, 2012). The perirhinal cortex (PER) activity has been related with both the detection of taste familiarity and attenuation of taste neophobia (Gómez-Chacón et. al., 2012). We have compared the taste neophobic response exhibited by three groups of rats: adult intact, adult PER-lesioned and aged (24-month-old). We have also assessed PER Fos-like immunoreactivity (FLI) while drinking either the novel or the familiar taste in aged rats. The results showed slower habituation of taste neophobia both in PER-lesioned and aged rats than in adult rats. Moreover, aged rats did not show the previously reported PER FLI increase related with taste familiarity. The results are discussed in terms of aging-related decay of PER function. Grant PSIC2011-23702 (MINECO. Spain), supported by FEDER funding.

## #P032 Metabotropic signal transduction in pheromone sensitive olfactory receptor neurons of the hawkmoth Manduca sexta

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In insect olfactory receptor neurons (ORNs) the involvement of the olfactory coreceptor Orco in the transduction of odor information is still under discussion. With extracellular tip recordings from pheromone-sensitive trichoid sensilla we examined the role of Orco in pheromone transduction of the hawkmoth Manduca sexta. Application of the Orco agonist VUAA1 did not increase the fast, phasic responses to the pheromone component bombykal (BAL). However, it increased the activity of ORNs within seconds to minutes after BAL stimulation as well as in the absence of pheromone. This suggests that BAL-transduction in M. sexta is not mediated via Orco-based ionotropic mechanisms. Rather Orco forms a pacemaker channel which controls the ORNs resting potential. Consistent with this hypothesis interference with the PLCβ-cascade changed pheromone responses and spontaneous activity of hawkmoth ORNs. Furthermore, ELISAs of M. sexta antennae found daytime-dependent changes of antennal IP3 levels. Thus, our data suggests that in hawkmoths pheromone transduction activates the PLCβ pathway resulting in second messenger-dependent modification of ion channels such as the slow Orco pacemaker channel. [Supported via DFG SPP 1392, STE 531/20–1 to MS]

## #P033 Regulation of pulse resolution in insect ORs: from molecule to behavior

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The detection and resolution of brief, repeated filaments in a plume is essential for flying insects to locate an odor source. We applied genetic, physiological and behavioral analyses to investigate mechanisms underlying pulse resolution and sensitivity at the insect periphery. We show that odorant stimulation of odorant receptor (OR)-expressing OSNs increases PKC activity. Disrupted Orco phosphorylation by PKC reduces the response to brief pulses, as well as pulse resolution of low odor concentrations. A single mutation of either PKC53E or PKCδ also reduces OSN sensitivity and response to brief stimuli, showing the importance of PKC for the odor response. In contrast, stimulation of ionotropic receptor-expressing OSNs does not change PKC activity and mutation of PKC genes does not affect their odor response. Finally, flies with inhibited PKC activity do not respond to brief odor pulses in behavioral assays and have difficulty locating an odor source in free flight assays. Using our comprehensive approach, we conclude that modulation of ORs by PKC contributes to both sensitivity and speed of odor detection to enable flying insects to quickly follow dynamic odor plumes. Supported by the Max Planck Society and the DFG (SPP 1392).

## #P034 Intrinsic oscillatory discharge patterns in mitral cells of the mouse accessory olfactory bulb

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The accessory olfactory bulb (AOB) represents the first stage of central information processing in the rodent accessory olfactory system. Mitral cells (MCs), the main excitatory projection neurons in AOB, receive input from vomeronasal sensory neurons, and project directly to higher brain regions such as amygdala and hypothalamus.Here, we investigate the biophysical properties of AOB MCs in acute mouse AOB slices. Using electrophysiological and pharmacological approaches we identify an MC population that displays slow oscillatory discharge patterns which persist after pharmacological inhibition of synaptic transmission. The underlying subthreshold membrane potential fluctuations display a high degree of periodicity that is mediated by multiple voltage-gated ion channels such as TTX-sensitive Na+, TEA-sensitive K+, and SNX-sensitive Ca2+ channels.The observed oscillations could play an important role in the signal coding and / or hormonal homeostasis controlled by MC target regions in higher brain centers. The work is supported by the DFG SPP 1392

## #P035 Olfactory cortical neurons read out a relative time code in the olfactory bulb

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Odor stimulation evokes complex spatiotemporal activity in the olfactory bulb, suggesting that the identity of activated neurons as well as the timing of their activity convey information about odors. However, whether and how downstream neurons decipher these temporal patterns remains debated. We addressed this question by measuring the spiking activity of downstream neurons while optogenetically stimulating two foci in the olfactory bulb with varying relative timing in mice. We found that the overall spike rates of piriform cortex neurons were sensitive to the relative timing of activation. Posterior piriform cortex neurons showed higher sensitivity to relative input times than neurons in the anterior piriform cortex. In contrast, olfactory bulb neurons rarely showed such sensitivity. Order sensitivity did not depend on the time relative to the sniff phase of optical stimulation. This strongly supports a relative time coding scheme in the olfactory system. We conclude that the brain can transform a relative time code in the periphery into a firing-rate-based representation in central brain areas, providing evidence for the relevance of relative time-based code in the olfactory bulb.

## #P036 TRPM3 – A promising target for analgesic treatment

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The superfamily of Transient Receptor Potential (TRP) ion channels consists of 28 different members in mammalians. The sensitivity of TRP channels to a broad array of stimuli allows them to function as biological sensors involved in processes ranging from vision to taste, and tactile sensation. The so-called thermoTRPs (temperature sensitive) are typically expressed in sensory neurons, where they act as primary thermosensors for the detection of innocuous and noxious temperatures. Lately, our research group identified high TRPM3 expression in nociceptor neurons, where it plays a decisive role in the nocifensive response to pregnenolone sulphate and heat and in the development of heat hyperalgesia during inflammation.This project aims to validate TRPM3 as a potential target for the development of new analgesics. Therefore, we want to identify new potent, selective TRPM3 blockers, show their ability to cure pain conditions in vivo and use them as tools to illuminate the role of TRPM3 in different pain pathways. Our first results show a potent and highly selective TRPM3 blocker able to treat inflammatory pain in mice. The current project may provide a basis for the development of TRPM3 antagonists for use as analgesics in humans.

## #P037 Zebrafish crypt neurons project to a single, identified mediodorsal glomerulus

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Crypt neurons are a third type of olfactory receptor neurons with a highly unusual "one cell type - one receptor" mode of expression, the same receptor being expressed by the entire population of crypt neurons. Attempts to identify the target region(s) of crypt neurons have been inconclusive so far. We report that TrkA-like immunoreactivity specifically labeled somata, axons, and terminals of zebrafish crypt neurons and reveal a single glomerulus, mdg2 of the dorsomedial group, as target glomerulus of crypt neurons. Injection of a fluorescent tracing dye into the mdg2 glomerulus retrogradely labeled mostly crypt neurons, as assessed by quantitative morphometry, whereas no crypt neurons were found after injections in neighboring glomeruli. Our data provide strong evidence that crypt neurons converge onto a single glomerulus, and thus form a labeled line consisting of a single sensory cell type, a single olfactory receptor and a single target glomerulus.

## #P038 Comparative analysis of G-protein coupled receptor trafficking to the plasma membrane

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Every member of the large family of odorant receptors (ORs) has the particularity to not express successfully in vitro. When transfected into cell lines, they surprisingly don't traffic to the plasma membrane and remain in the Endoplasmic Reticulum (ER) and eventually degraded. Our goal is to determine why all ORs don't traffic to the plasma membrane, while other G-Protein Coupled Receptors in the same class, like the β2-adrenergic receptor (β2AR), can robustly traffic. In this study, we have begun to break down the biological barriers that are preventing plasma membrane expression of ORs by understanding how GPCRs traffic to the plasma membrane. We have performed an extensive and unique analysis of plasma membrane trafficking of the OR M71 and that of the canonical GPCR, the β2AR. Our efforts can be divided into two approaches, we have tried to "fix" M71 trafficking through targeted mutagenesis and chimeric analyses with β2AR, and we have "killed" the β2AR through targeted mutagenesis and chimeric analyses with M71. We

have determined that OR retention in the ER is a result of distributed features of the OR sequence; it is not one region that is preventing functional expression at the plasma membrane in heterologous cells.

## #P039 Puberty-accelerating male pheromones induce early expression of male-directed odor preference in adult female mice

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Puberty onset in female mice is accelerated by male pheromones. Whether peripubertal exposure to male odors also influences female sexual behavior in adulthood is at present unclear. We provide evidence that females exposed to male odors and thus showing puberty acceleration, displayed a preference for intact male over female or castrated male odors at postnatal day (PD) 45; while a preference for male odors emerged later (at PD60) in control females. By GC/MS analysis, we found several volatiles in male-soiled bedding known to induce puberty acceleration, such as 6-hydroxy-6-methyl-3-heptanone (HMH), 2-sec-butyl-4,5-dihydrothiazole (SBT) and 3,4-dehydro-exo-brevicomin (DHB) and we replicate previous results showing that peripubertal exposure to HMH, SBT or DHB accelerated the onset of puberty in female mice. In addition, we show that exposure to the blend of these three molecules induced early expression of male-directed odor preference at PD45, contrary to the single exposure to each of these molecules. In conclusion, male pheromones exposed during the peripubertal period influence both female puberty and adult expression of male-directed odor preference.I thank ECRO to support my attendance to this meeting.

## #P040 Individual physiological responses to odors and their relationship with nutritional status in young and old adults

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The link between emotional odor perception and peripheral nervous system activity is well documented, but little is known about the individual variability of these responses. Aims of this study were to characterize this variability and to examine the relationship between physiological responses to odors and the nutritional status. To achieve these aims, 20 young and 20 old subjects rated odor pleasantness, intensity, familiarity and edibility of 18 odorants and completed the Mini Nutritional Assessment (MNA). Multiple physiological measures, including skin conductance and corrugator facial electromyogram, were recorded continuously. Results revealed a high variability in the distribution of individual correlation coefficients (r-values) between perceptual and physiological parameters. Regression between those r-values and MNA scores revealed that old but not young subjects who had appropriate physiological responses to intense, unpleasant and non-edible odors were also those who had better nutritional status. Taken together, preserving appropriate functioning of somatic markers in response to odors during normal aging is associated with better nutritional state, and is likely to facilitate healthier food selection.

## #P041 Analysing the role of miR-200 in zebrafish taste bud formation

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Taste bud cells are induced in specific sites of the oropharyngeal epithelium (placodes) upon activation of Wnt/beta-catenin signaling and subsequent Sox2 expression. Wnt/beta-catenin signaling activates the Shh and Bmp signaling pathways that contribute to appropriate patterning of taste buds within the oropharynx (1). Although several key molecules that are necessary for taste bud induction have been identified, little is known about the signals that regulate taste bud cell differentiation. We have recently shown in zebrafish that the miR-200 family is required for taste receptor cell formation (2). However, the mRNA targets of miR-200 in this process remain unknown. Through RNAseq, we compared mRNA expression in dissected pharynges from control and MO-miR-200 family injected embryos. We then compared these results with the bioinformatically predicted miR-200 targets. A handful of selected genes is further analyzed and will be discussed in the meeting.1.Semin Cell Dev Biol. 2012 Nov 24. doi:pii: S1084-9521(12)00204-2.2.Development 2011 138:3473–84.Funding: ANR-09-BLAN-077, Fondation pour la recherche medicale.

## #P042 An analysis of sour responses in clonal cell lines derived from murine taste buds using calcium imaging method

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We have recently established a number of clonal cell lines from taste buds of a *p53*-deficient mouse (TBD cell lines). Several taste receptor molecules were observed in TBD cell lines by RT-PCR. We examined whether TBD cell lines respond to taste stimuli using calcium-imaging analysis. A transient elevation of $[Ca^{2+}]_i$ was elicited by sour taste stimuli in TBD cell lines. TBD cell lines more strongly responded to citric acid than HCl and showed pH-dependency. Responsiveness to sour stimuli was not influenced by reduction in extracellular $Na^+$ and $Ca^{2+}$ concentration, and application PIK-3 or PLC-β2. These results were consistent with properties of sour response through PKD1L3/2L1, suggesting that these TBD cell lines are useful as in vitro model for study of sour transduction.

## #P043 Generalization of experience-induced changes in taste sensitivity for sugars and non-nutritive sweeteners suggests an overall change in receptor responsiveness

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Human psychophysics and animal neurophysiology data suggest that experience-induced changes in taste sensitivity involve peripheral mechanisms, in or before the receptor cells. We have proposed that binding of the treatment compound with the receptor subunit T1R3 leads to changes in the receptor response to the test compound. We studied the effects of treatment with Na-cyclamate (Na-c), which binds T1R3, on human taste sensitivities for the monosaccharides glucose (pyranose) and fructose (furanose), the disaccharides maltose (pyranose) and sucrose (furanose), and the structurally different non-nutritive sweeteners, Na-c, sucralose and D-tryptophan. Subjects rinsed their tongues with 4 mM Na-c or water for 10 sec once a day for 10 days. On day 11 or 12, they tasted a concentration series of the test stimulus, each concentration paired with water, and chose which of each pair was 'the sweetener.' Sensitivities for glucose, fructose and maltose were increased ($p=or< 0.004$). They were increased for Na-c and sucrl ($p= 0.03, 0.004$) and decreased for D-tryp ($p=0.006$). Since MSG sensitivity also is decreased after Na-c, the effect may differ for amino acids. The generalization of effects supports changes in the overall receptor response.

## #P044 The OR37 subfamily: a special olfactory subsystem

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Members of the OR37 subfamily differ from other vertebrate odorant receptors due to a variety of special features. They are exclusively found in mammals, highly conserved during evolution and exhibit a unique structural element, indicating that OR37 receptors are tuned to special ligands. Distinct mouse OR37 subtypes can indeed selectively be activated by different long-chain aliphatic aldehydes, which are produced by conspecifics. OR37-specific projection neurons are wired to higher brain centers in an atypical manner: they are connected to the medial amygdala, a brain area involved in processing social cues, and to the hypothalamus, a central neuroendocrine regulator. Altogether the data indicate that the OR37 subsystem serves a special function in mammals. Towards an understanding of its functional role, neurophysiological and behavioural analyses are performed upon activation of OR37 expressing cells.

## #P045 Responses to predator odors are modulated by early olfactory experience in the house mouse

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Neonatal exposures to odorants influence the function of the olfactory system to produce changes in responses to these stimuli later in life. Modulation of olfactory sensitivity by environmental factors as well as induction of sensitivity to some odorants considerably influences an organism's adaptability. To further explore the phenomenon of sensitization, we manipulated odor environment of developing mice. We exposed mice Mus musculus during days 14–28 after birth to the urine from natural predator – domestic cat; species specific compound from the cat urine – L-felinine(0.05%) or tap water. Olfactory thresholds were

assessed at age of two months using a multi-stage olfactometer (Knosys). Hormones were measured using EIA. Orienting-investigatory activity in presence/absence of predator chemical cues was recorded in open field test. Early neonatal exposures to cat urine/felinine significantly ($p< 0.01$, n=25) lowed behavioral olfactory thresholds. A number of indicators of orienting-investigatory behavior was modulated by early exposures to cat urine/felinine (n=20, $p<0.05$). At the same time corticosterone response to felinine (0.05%) or predator chemical cues from cat urine stayed unchanged. Supported by MK-709.2012.4

## #P046 Androstenone exposures affect plasma testosterone level and behavior in mice

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Chemical signals are involved in regulation of social behaviors in mammalian species. Mice engage in anogenital and/or naso-oral investigation prior to initiating either sexual advances towards female or aggression with an unfamiliar male. Excreted steroid hormone metabolites may be considered as putative pheromones since they convey information about sex, physiological status and social rank of individual. As a model we studied signal role of sex boar pheromone androstenone (AND) in the House Mouse. Exposures of CBA/Lac (CBA) males to AND (0,1%; 30 min) suppressed basal testosterone plasma levels ($p<0,05$, n=15) as well as blocked testosterone surge caused by presentation of estrus female urine ($p<0,001$, n=16). Exposures to AND (0,025%;30 min.) also affected behavior of CBA males (n=27). In odor preference test CBA males usually show preference for estrus female odor vs male odor. We did not observe the above after AND exposure. In the open field test AND exposure resulted in an increase of vertical activity ($p<0,05$) and in a decrease of grooming behavior ($p<0,01$). The data obtained indicate that AND known as sex boar pheromone may show pheromonal activity in mice. Supported by RFBR 12-04-32079 and MK-709.2012.4.

## #P047 A binary genetic strategy to visualize and manipulate TRPM5-expressing cells in mice

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TRPM5 is a voltage-modulated and calcium-activated monovalent cation channel highly expressed in mouse taste tissue. It is a fundamental signaling component of type II taste receptor cells and genetic deletion leads to major deficits in sweet, bitter and umami sensation. Recent studies show that TRPM5 expression is not restricted to the tongue but that it is also present in a variety of chemosensory cells distributed throughout the body. To gain further insights into TRPM5 expression and its function in various mouse tissues we developed a binary genetic approach to visualize and manipulate TRPM5-expressing cells. Towards this end, we have generated a novel knock-in mouse strain which expresses Cre recombinase in TRPM5-expressing cells. We will present data showing that Cre-mediated recombination results in direct activation of a reporter allele in TRPM5-expressing cells which allows their visualization at single cell resolution in vivo.

## #P048 Reception of sex pheromones in the house mouse is modulated by glucocorticoids

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The role of sex hormones in perception and analysis of chemical cues are studied very well, while the role of stress hormones remains unclear. We compared the effects of acute vs chronic stress on the reception of sex pheromones in mice. Extended exposure to emotional stress affected performance of males in standard odor preference test. Males did not prefer odor of estrus female vs diestrus female (n=40, $p \leq 0.01$). Patterns of sexual behavior were decreased in standard pairing test (t=60 min., n=8, $p<0.05$). Number of Fos-positive cells in male VNO receptor epithelium (RE) in response to receptive female odor was significantly reduced in case of acute stress (n=8; $p<0.05$); Fos-IR was fully blocked in case of chronic stress exposure. Using pharmacological, IHC and endocrinological approach we have determined plasma corticosterone (CRT) concentration range sufficient to block males response to receptive female odor at the behavioral, hormonal and at the level of VNO RE:230-250ng/ml. Pharmacological analysis showed a lack of influence of indicated CRT levels on the olfactory memory retrieval processes, which points to peripheral mechanisms of the response suppression to receptive female odor in male mice. Funded MK -709.2012.4

## #P049 Modulations of olfactory responses by GAP junctions uncoupling in the olfactory mucosa.

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Glial cells, which are supporting cells of the nervous system, are now clearly involved in nervous communications. These cells modulate odorant detection as soon as the peripheral level in the olfactory mucosa, acting on olfactory sensitive neurons. These neurons

are surrounded by glial like cells called sustentacular cells. These cells maintain both the structural and ionic integrity of the olfactory epithelium and are electrically coupled through GAP junctions. Our study focused on endothelin known to activate glial cells and to uncoupled GAP junctions in the central nervous system. Using patch clamp, we first demonstrate that endothelin also uncoupled sustentacular cells in vitro. We then confirmed in vivo by calcium imaging that endothelin limits electrical signal propagation in intact olfactory epithelial preparation, similarly as the known GAP junction uncoupling carbenoxone. Such modulation could affect olfactory response kinetics as we observed that both endothelin and carbenoxone treatments increase EOG (Electro-OlfactoGramme) signal repolarization. Our results are thus consistent with an implication of glial cell like modulation of nervous information at the peripheral level of the nervous system.

## #P050 In vitro perception of the odor of lung cancer biomarker using bioelectronic nose

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Intractable diseases cause the composition of chemical metabolites in blood to change; however, insensitive human nose cannot perceive such changes. In this work, we developed a bioelectronic nose that mimics the function of human nose for the in vitro perception of the odor of lung cancer patient's blood. A specific olfactory receptor (OR) recognizing heptanal, a biomarker of lung cancer, was first determined through screening a library of human ORs, and was used for the functionalization of carbon nanotubes in a form of nanovesicle containing signaling components. The developed bioelectronic nose was able to selectively detect the biomarker in a femto-molar range, a sufficient level to distinguish patient's blood from non-patient's blood. Moreover, the signals generated from the bioelectronic noses were amplified by co-expressing two accessary proteins, human Gαolf protein and receptor-transporting protein 1S, which assist the olfactory signaling and high-level expression of ORs. The bioelectronic nose was able to recognize an extremely small increase in the amount of heptanal from human blood plasma without any pretreatment processes. This result offers a rapid and simple method to analyze odorous biomarkers from human blood.

## #P051 Effects of adiponectin on the olfactory system

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The peptide hormone adiponectin is secreted by adipose tissue and the circulating concentration is reversely correlated with body fat mass; it is considered as starvation signal. The observation that mature olfactory sensory neurons (OSNs) express the adiponectin receptor 1 has led to the concept that adiponectin may affect the responsiveness of the olfactory system. In fact, electroolfactogram recordings from olfactory epithelium incubated with exogenous adiponectin resulted in larger amplitudes upon odor stimulation. The application of adiponectin to the nasal cavity of mice led to an increase in the number of activated OSNs upon exposure to distinct odorants compared to control mice. Furthermore, the number of activated periglomerular neurons surrounding a receptor-specific glomerulus was higher in these mice indicating that the augmented responsiveness of OSNs was strong enough to elicit a higher neuronal activity in the olfactory bulb. The results of this study indicate that adiponectin increases the responsiveness of the olfactory system, probably due to a higher responsiveness of olfactory sensory neurons.This study was supported by Deutsche Forschungsgemeinschaft (DFG).

## #P052 Receptor basis for bitter perception in mice

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Phylogenetic analysis indicated that Tas2r clusters in mice exhibit syntheny with TAS2R-rich regions on human chromosomes. Several Tas2r genes within these regions are considered orthologs. However, it remains to be proven, if human and mouse sequence-orthologous Tas2rs represent functional orthologs.Quantitative RT-PCR found all murine Tas2rs expressed on the posterior tongue, indicating that they all function as bitter taste receptors. We challenged all 35 murine Tas2Rs with 140 bitter substances in functional assays and identified cognate bitter compounds for only 18 Tas2rs (51%) whereas we previously deorphaned 84% in humans. Mice and man possess generalists, moderately tuned Tas2rs and specialists based on the number of their cognate bitter compounds. However, mice have more specialists, proposing that a greater number of Tas2r genes offers the luxury of having specific receptors for solitary bitter compounds. Importantly, the data revealed that the two species mostly recognize the same compounds with non-orthologous Tas2rs.Apparently, the potential for dynamic adaptation of Tas2r functions to nutritional needs is more important than the simple fixation of their pharmacological properties.

## #P053 Long-term exposure to predator odors affects plasma testosterone in male rats

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Felinine is a unique sulfur-containing amino acid found in the urine of domestic cats and select members of the Felidae family. In our earlier studies we showed that exposures of rats Rattus norvegicus during gestation to the cat urine or

L-felinine suppressed reproductive output. In the current study we examined the influence of species specific compound L-Felinine vs intact cat urine on testosterone secretion in male rats. We used concentrations of L-felinine comparable with those naturally occurring in cat urine. Exposure of male rats to cat urine during two weeks significantly suppressed plasma testosterone ($p<0,05$, $n=10$). Analogous L-felinine exposures (0,05 %, 0,2ml) produced the very same effect ($p<0,05$, $n=10$) in male rats but we did not observe the effect when lower dose was used (0,05 %, 0,1ml.). The expression of the secondary defense reactions by laboratory naïve rats and the failure to habituate at the hormonal level indicates an innate nature of the response. We suggest that rats could assess the predator danger by the L-felinine/or derivates concentration in the cat urine. Supported by RAS Program «Live Nature»

## #P054 Plasticity of the glomerular maps in the mouse olfactory bulb

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In the olfactory bulb, odor stimulations evoke spatio-temporal patterns of activity. Plasticity of oscillatory activities has been shown by recording the local field potential during learning. However, very few is known concerning the plasticity of spatial coding, characterized by the activation of glomerular maps. In vivo intrinsic optical imaging is a technique based on changes of endogenous optical properties of the brain tissue during activation. We used this technique to compare glomerular maps obtained for the same odors before and after an operant olfactory conditioning task. Mice were implanted with a chronic cranial window centered on the olfactory bulb and activity was recorded under anesthesia. One group of animals was trained to solve an odor discrimination task (go-nogo task; paired condition). For another group, odors were randomly associated with the reinforcement (unpaired condition). We observed no change of odor responses in the paired group. In the unpaired group, the spatial maps were not modulated by the odor stimulation, but activation was decreased for the odors mice were exposed to. Our data demonstrate an impact of the animal's experience on the spatial dimension of odor representation.

## #P055 Developmental expression of TMEM16A and TMEM16B in the mouse olfactory epithelium

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Developmental expression of TMEM16A and TMEM16B in the mouse olfactory epithelium.DEVENDRA KUMAR MAURYA and ANNA MENINILaboratory of Olfactory Transduction, SISSA, International School for Advanced Studies, Via Bonomea 265, 34136 Trieste, ItalyTMEM16A/anoctamin1 and TMEM16B/anoctamin2 have been recently identified as responsible for calcium-activated chloride currents. They are both expressed in the olfactory epithelium, but little is known about their expression during embryonic development. We have studied by immunohistochemistry the expression of TMEM16A and TMEM16B in the olfactory epithelium (OE) at various stages of prenatal development (E12.5-E18.5) and in neonatal mice. We found that TMEM16A is expressed in the apical layer of the olfactory epithelium at E12.5, while the onset of expression for TMEM16B is E14.5 with an increase in expression in subsequent days. Co-localization experiments show that TMEM16A and TMEM16B are expressed in two different layers, with TMEM16B in the uppermost layer. Moreover, TMEM16A is not expressed in olfactory sensory neurons, while TMEM16B is localized to the cilia of olfactory sensory neurons, consistent with a role in olfactory transduction.

## #P056 A paper screening test to assess oral sensitivity to oleic acid in normo-weight PROP super-tasters, medium tasters and non-tasters

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Several works reported that PROP non-tasters are less responsive to various oral sensory qualities, including fats, and have a lower ability to distinguish fat content in foods. Other reports did not confirm these associations. Besides, a difficult problem in the investigation of human oro-sensory responses to free fatty acids (FFAs), is that of creating homogenous and stable oil-in-water emulsions. We developed a method that bypasses this difficulty and measures the oleic acid detection thresholds in normo-weight PROP super-tasters, medium tasters and non-tasters. Cognitive eating behaviours and BMI were determined in 47 subjects classified for their PROP taster status. Thresholds for oleic acid were assessed by a modification of the 3-alternative forced-choice procedure where stimuli were presented in filter paper disks. Thresholds for oleic acid were higher in PROP non-tasters relative to the other groups, and the restraint score was inversely correlated to oleic acid threshold and directly to BMI. Our results indicate that the paper screening test is a valid and reliable method to assess oral sensitivity to FAs, and show a direct relationship between PROP and oleic acid sensitivity, and between restraint and FA sensitivity.

## #P057 Leg chemoreceptor sensitivity in the red swamp crayfish Procambarus clarkii

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The red swamp crayfish Procambarus clarkii (Crustacea: Decapoda), one of the most invasive species of freshwater habitats, relies on chemical cues to locate food resources. Here we investigate the sensitivity spectra of the walking leg chemoreceptors to different compounds of possible feeding significance in relation to the omnivorous habits of P. clarkii.Extracellular nerve recordings showed that the disaccharides trehalose and cellobiose were the most stimulating sugars for leg chemoreceptors, but to a different extent: an extremely phasic time course for trehalose, more tonic for cellobiose. They were also comparable in stimulating effectiveness with the amino acids leucine and glycine (phasic-tonic responses), with different chemoreceptors being tuned to single compound classes. Leg chemoreceptors were also responsive to the monosaccharides glucose and fructose, taurocholic acid and, to a lesser extent, asparagine. Behavioural trials confirm the electrophysiological observations and assign an attractive role to all tested compounds. Such information may be helpful in the identification of key chemicals for crayfish, also aimed at the future development of strategies for population control programmes.

## #P058 The mouse Grueneberg Ganglion: an olfactory subsystem to detect danger

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The mouse olfactory system senses the odorants and pheromones present in the chemical environment. It is subdivided in four olfactory subsystems, which are the main olfactory epithelium (MOE), the vomeronasal organ (VNO), the septal organ (SO) and the Grueneberg ganglion (GG). The GG, focus of our studies, has been proposed to mediate the alarm pheromone (AP) detection in mice. Recently, we have been able to identify the precise chemical structure of one mouse AP, the SBT (2-sec-butyl-thiazoline). Since then, we have also identified several new ligands activating the mouse GG neurons. They are very closely related to the identified mouse AP. They share similar chemical features as the sulfur-containing volatiles that are released by predating carnivores. Calcium imaging experiments help us to determine the molecular receptive range of GG neurons, allowing further comparisons with the chemical properties of known neurons expressed in other olfactory subsystems. We are now trying to identify the source of AP production. Moreover, molecular and immunohistochemical techniques have allowed the identification of multiple signaling elements in mouse GG neurons but their implication in the AP transduction pathway has to be demonstrated.

## #P059 Post-natal unilateral naris occlusion in mice induces olfactory receptor-specific plasticity at the cellular level

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In order to study olfactory peripheral system adaptation during development, we worked on a model of sensory deprivation by unilateral naris occlusion (UNO). UNO was performed by cauterization at birth of MOR23-IRES-tauGFP and M71-IRES-tauGFP mice. After 21 days, anatomical and molecular changes were analyzed in the open and closed side compared to untreated control animals. GFP-expressing neurons were counted from fixed and flatmounted epithelia. UNO induced a significant decrease of MOR23-expressing neurons density on the closed side while the density of M71-expressing neurons on both sides was not affected. Molecular study consisted of analyzing the mRNA expression level of olfactory receptors and central components of the transduction pathway through qPCR analysis. Dissociated GFP-expressing neurons were individually harvested and pooled by group of 4 to 8 for qPCR. The mRNA expression levels of AC3, CNGA2, PDE1C and MOR23 or M71 were monitored and variations were observed between open and closed sides. UNO effects are different in MOR23 and M71 neurons. Our results demonstrate a receptor-specific plasticity occurring after olfactory deprivation or over-stimulation in the peripheral olfactory system of developing mice.

## #P060 Gustatory thalamus and taste familiarity in rats

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In order to investigate the involvement of the gustatory thalamus in taste familiarity, Fos-like immunohistochemistry (FLI) was examined as an index of neural activity during attenuation of neophobia. The number of Fos-like positive cells in the parvocellular ventral posteriormedial nucleus of the thalamus (VPMpc) was examined in male Wistar rats during the first exposure (Novel group), the second exposure (Familiar 1 group) and after six exposures (Familiar 6 group) to a (3%) cider vinegar solution. The results showed that drinking a familiar taste solution induced a higher FLI in VPMpc than drinking a novel taste solution. Furthermore,

six exposures to the taste solution induced a higher FLI than two exposures. No differences were seen in other thalamic relay nuclei. Thereby, the results indicated that the neural activity of VPMpc depends on the level of taste familiarity, thus suggesting the involvement of VPMpc in the neural circuit of safe taste recognition memory. Supported by grant PSIC2011-23702 (MINECO. Spain). E. Morillas is recipient of an ECRO 2013 travel grant.

## #P061 Getting older: does olfaction fade away? A behavioural approach to mice third age

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In terms of mammalian evolution, olfaction plays a key role for individual survival, by detecting different types of odours as an alert on the presence of food, and as a warning for dangers such as predators, potential fire or even certain poisons. On the other hand, this sense is critic for species survival, allowing mate selection, favouring parental care in a herd or group of animals, and establishing territories. In this study we collect preliminary data on mice behaviour comparing two groups of old animals aged 13 months and 19 months. Tests evaluated motor skill, memory, aggressiveness and olfactory function, to assess their potential decline with age. We found some differences across groups. Firstly, a reduction in motor skills has been observed in older mice, while olfaction seems to be well preserved. Furthermore, an increase in aggressive behaviour was recorded in the older group. These results suggest that mice exhibit a reduction in awareness of dangers as a consequence of the aging process, allowing them to interact and survive more promptly with the environment.

## #P062 Selection on male sex pheromone composition drives butterfly diversification

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Natural selection facilitates diversification by inducing character displacement in mate choice traits that decreases the risk of maladaptive mating between lineages. Although reproductive character displacement has been demonstrated in two-taxa case studies, its frequency in nature is still debated. Using a group of 32 Bicyclus species, a largely sympatric African butterfly genus showing little morphological differentiation, we show recurrent reproductive character displacement and rapid evolution in predicted male sex pheromone composition, but not in other morphological mate choice traits. Sex pheromone evolution is unlikely due to the alternative processes of ecological character displacement or differential fusion. These results suggest that selection on divergence of sex pheromones contributed to the diversification within Bicyclus and that olfactory communication may play a more important role in species diversification than previously envisaged.

## #P063 The scent of inbreeding: male sex pheromones betray inbred males

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Inbreeding depression results from mating among genetically related individualsand impairs reproductive success. The decrease in male matingsuccess is usually attributed to an impact on multiple fitness-related traitsthat reduce the general condition of inbred males. Here, we find that the productionof the male sex pheromone is reduced significantly by inbreeding inthe butterfly Bicyclus anynana. Other traits indicative of the general condition,including flight performance, are also negatively affected in malebutterflies by inbreeding. Yet, we unambiguously show that only the productionof male pheromones affects mating success. Thus, this pheromonesignal informs females about the inbreeding status of their mating partners.We also identify the specific chemical component, hexadecanal, probablyresponsible for the decrease in male mating success. Our results advocategiving increased attention to olfactory communication as a major causalfactor of mate-choice decisions and sexual selection.Published in the Proceedings of the Royal Society of London (2013) in press.

## #P064 Genetic dissection of the olfactory mediated aggression in male mice using Mup genes network: methodological consideration, current status, and prospects of the model

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Aggressive behavior takes place between at least two animals and is realized through extremely complex exchange of information between partners via several sensory modalities [Novikov, 1993]. For mice the leading sensory

modality is olfaction, and much of the information is conveyed by pheromones, which have the ability to initiate innate (genetically programmed) behavioral responses. In previous work we suggested that ratios and concentrations of the physiologically active major urinary proteins (MUPs) [Chamero et al., 2007] of the given combination define behavioral response of the recipient mouse [Novikov et al., 2009]. Taking into account recent developments in this field [Cheetham et al., 2009; Chamero et al., 2012; Boehm, 2013], our model can effectively outline complex chemosensory pathways from specific genes to olfactory mediated physiological responses via sensory neurons of the vomeronasal organ. My talk will summarize recent data on the key role of MUPs in social recognition in Mus musculus L. and highlight the advantages of using Mup genes network in genetic dissection of the intermale aggressive phenotypes in this species [Fedorova et al., 2011]. Supported by RFBR (projects 02-04-49273, 04-04-63050, 07-04-01762)

## #P065 Activation properties of TAS2R14, a bitter taste receptor with an exceptional wide agonist spectrum

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The 25 TAS2Rs belong to the G protein-coupled receptor family mediating bitter taste perception in humans. The TAS2R14 is one of three very broadly tuned receptors and responds to important drugs, powerful toxins as well as frequently encountered dietary bitter compounds.To investigate the binding site of TAS2R14 we performed alanine-scanning mutagenesis and measured the effects of these changes by functional calcium imaging assays. The results on critical receptor positions were used to develop and successively refine a homology model of TAS2R14 and to perform in silico agonist docking studies. To confirm and extend the results obtained from in silico studies, additional, more subtle, receptor mutants were generated and functionally analysed. In the course of these experiments we identified for several receptor mutants pronounced agonist selective effects. The observed effects ranged from selective losses of function to improvements of receptor responses. Intriguingly, mutagenesis of residues, W66, W89, and N93 leading to losses of function in other TAS2Rs, only decreased TAS2R14 agonist activation. An advanced model of the receptor together with experimentally validated agonist docking results will be presented.

## #P066 Coupling ion channels with olfactory receptor for highly sensitive sensing of odorant

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In mammalian cell-based system, various signal detection methods using intracellular mechanism have been applied for odorant screening. The detection of calcium influx is the one of the widely used methods. But this detection system is time-consuming and labor-intensive and also it needs secondary signal messenger generated via complex signaling cascade upon ligand stimulation. In this study, we adopted the concept of ion channel-coupled receptors (ICCRs) for the detection of ligand binding by direct ionic current change using membrane potential dye. We coupled the human olfactory receptor hOR2AG1 with the potassium channel Kir6.2 and expressed the fusion protein in HEK293 cells. The ionic current change upon ligand stimulation was detected with spectrofluorometer and fluorescence image scanner. And the cells expressing hOR2AG1-Kir6.2 showed considerably higher membrane potential change than those expressing only OR2AG1. This result showed that hOR-Kir6.2 can be used for a simple and effective sensing in the mammalian cell-based assay system. Moreover, this label-free sensing material can be used as promising tools for a high-throughput odorant screening system.

## #P067 Screening for and characterization of pharmacological tools to target the Ca2+ activated non-selective cation channels TRPM4 and TRPM5

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TRPM4 and TRPM5 are monovalent cation selective ion channels, which are activated by an increase of the intracellular Ca2+ concentration. Physiologically, TRPM5 is a key player in the transduction of taste signals and plays a role in the release of insulin from pancreatic ß-cells. TRPM4 is important for tuning the activation state of mast cells, and might be a novel drug target for allergic diseases. To date no pharmacological tools are available to evaluate their potential as drug targets.In this study we present a screening method for identifying compounds, which target these channels. We used a fluorescence based high throughput device to visualize intracellular [Na+] dynamics to screen an extensive library of compounds (> 10.000). Furthermore we have characterized novel activators of TRPM5 using patch clamp studies. These compounds are selective for the TRPM5 channel, and have a direct effect on the channel. We used calcium imaging in pancreatic islets of Langerhans to asses the effects of the compound in a physiological context and

could observe an enhanced activity.Potentially it could play an important role in the development of new taste modulators, and novel treatments for type 2 diabetes.

## #P068 Changes in olfactory bulb volume following lateralized olfactory training

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Olfactory training plays a significant role in the treatment of hyposmia. It is known that repeated exposure to odors modifies olfactory function. In addition, numerous studies show that the olfactory bulb (OB) volume changes under various conditions. The aim of this study was to investigate whether the OB volume changes in relation to lateralized olfactory training.Over a period of 4 months 97 healthy participants (34 m, 63 f) performed olfactory training. They were exposing the same nostril twice a day to 4 odors (lemon, rose, eucalyptus and cloves) while closing the other one. Before and after the training, MRI scans were performed to measure the OB volume. Furthermore, participants underwent lateralized threshold and identification testing using the "Sniffin Sticks" test.Although there was no significant change in olfactory function comparing results before and after training, it was found that the OB volume increased significantly by 11.3% in the trained nostril and 13.1% in the untrained nostril. No significant effect was found comparing the influence of gender, age, duration and frequency.These data indicate that olfactory training induces a top-down process which ultimately leads to a bilateral increase in OB volume.

## #P069 Long-term plasticity at the olfactory bulb mitral – granule cell synapse

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In the mammalian olfactory bulb, axonless granule cells (GCs) mediate self- and lateral inhibitory interactions between mitral cells (MCs) via reciprocal dendrodendritic synapses. MC activity during odor sensation occurs in repetitive bursts that are synchronized to the respiration rhythm. We began to explore long-term plasticity at this synapse by using a theta-burst type protocol (TBS, five 40 Hz bursts at 4 Hz, ten times at 0.1 Hz) for glomerular stimulation during whole-cell recordings from GCs in acute brain slices from juvenile rats. The TBS protocol reliably induced long-term depression (LTD) of granule cell EPSPs in most cells tested ($81 \pm 18$ % of control, $n = 16$, $P < 0.002$), whereas theta stimulation alone ($n = 8$) or a train of 40 Hz stimulations equal in number to the TBS protocol ($n = 6$) were ineffective ($P > 0.05$). Since rats can detect and discriminate odors within single sniffs, we have also applied a "single sniff" paradigm based on a single 40 Hz burst (SBS). Subsequently, either LTD or LTP were observed ($n = 21$). SBS plasticity was "homoglomerular" ($n = 6$). Blockade of NMDA receptors prevented both TBS- and SBS-induced plasticity ($n = 6$, $n = 5$). Thus in-vivo-like MC activity can cause substantial plasticity.

## #P070 The capsaicin-analog nonivamide prevents lipid accumulation via a TRPV1-dependent pathway and regulates related miRNA expression in 3T3-L1 cells

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Activation of the TRPV1-receptor is not only responsible for the pungent effects of capsaicin (CAP), but is also known to induce molecular mechanisms of lipid metabolism that counteract adipogenesis and obesity. We investigated whether a less pungent structural analog of CAP, nonivamide (NV), has similar effects on lipid accumulation in 3T3-L1 cells and whether TRPV1 receptor activation and miRNA regulation is involved in the underlying pathways. Addition of CAP or NV to 3T3-L1 cells in a concentration range of 1 nM to 10 µM equally reduced lipid accumulation during differentiation and maturation by up to $10.1 \pm 4.98$% and $10.5 \pm 7.81$%, resp. This effect was abolished by co-incubation with CAP or NV and the TRPV1-inhibitor trans-butylcyclohexanol. miRNA analysis of mature miRNAs showed that treatment with 10 µM NV down-regulated, e.g., miR-103, in comparison to non-treated control cells. qPCR analysis of miR-103 target genes revealed a down-regulation of PPARγ after NV treatment.In conclusion, our data demonstrate that the less pungent TRPV1 agonist NV has similar effects on adipogenesis as CAP, and suggest an involvement of NV on TRPV1 receptor activation and miRNAs regulation as part of anti-obesity mechanisms in 3T3-L1 cells.

## #P071 Perception of blending mixtures at different ages – A pilot study in humans

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Complex stimuli can be processed either by extracting information about the elements or as a whole (elemental vs configural perception). In vision, it has been suggested that such perceptual abilities depend in part on age; young individuals would rely less on configural processing. In olfaction, configural perception is observed in blending mixtures which evoke a specific percept different from the odorants. For instance, odorants A and B which respectively evoke a strawberry and a caramel odour are well perceived by adults within the AB mixture at a non-blending ratio (50/50). However, at a 30/70 ratio, human adults perceive a pineapple configural odour. Similarly, a senary mixture smells like red-cordial (RC) while none of its components is typical of that quality. Here, we compared how child, teenagers and adults (10, 14 and 43 y.o., respectively) submitted to a complexity and a typicality rating tasks, perceived the AB and RC mixtures. For the RC mixture, ongoing analyses show no difference between age groups whereas child and teenagers only perceive the AB blending mixture as less complex than the non-blending one. These results suggest that mixture processing varies as the function of age for certain mixture compositions.

## #P072 Time-frequency analysis after chemosensory stimulation

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Time-frequency analysis constitutes an alternative approach to reveal activity that is induced by a chemosensory stimulus, but not sufficiently stationary across trials to be revealed by classic averaging in time-domain. In this way, it could increase the signal-to-noise ratio of chemosensory EEG responses. Different methods exist to perform a time-frequency decomposition of EEG epochs. These methods rely on techniques to estimate within each EEG epoch the amplitude of the signal as a function of time and frequency, regardless of the phase. The obtained time-varying expressions of oscillation amplitude are then averaged across trials, thereby disclosing both phase-locked and non-phase locked modulations of signal amplitude, provided that these modulations are relatively well time-locked to the onset of the event, and consistent in frequency. We have recently shown that the time-frequency approach markedly improved the signal-to-noise ratio of the EEG responses to chemosensory stimulation (in particular following olfactory stimulation), in comparison to conventional time-domain averaging.

## #P073 Expression of the nutrient sensor family Tas1R along the porcine gastrointestinal tract

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The 3 members of the GPCR T1R receptor family are known to form heterodimers that are responsible for the sweet (T1R2 + T1R3) or umami (T1R1 + T1R3) tastes. Studies in rodents and humans have established that Tas1R genes are expressed in the tongue, but also outside of the oral cavity. However, only a few studies have reported Tas1R expression in porcine gastrointestinal tract (GIT) tissues. The aim of this study was a systematic analysis of the expression of the Tas1R gene family in 12 different tissue samples (3 tongue papillae, 2 stomach -ridge and antrum-, duodenum, ileum, jejunum, 2 colon -proximal, distal-, caecum and liver) by real-time PCR. Regardless of tissue, the expression level of Tas1R3 was higher than both Tas1R1 and Tas1R2. The Tas1R2 was only significantly expressed in liver and tongue. In particular, the expression of Tas1R2 and Tas1R3 was higher in circumvallate than in fungiform papillae. This suggests that pigs may have a higher capacity to taste sweet in the back of the tongue. Outside the oral cavity, the two stomach tissues had higher expression levels of Tas1R1 and Tas1R3 than the other tissues analysed. Our results demonstrate that pigs may have a specific pattern of expression of Tas1Rs in the GIT.

## #P074 PLC and TRPC2 mediate amino acid detection in the main olfactory epithelium of an amphibian

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Most amphibians have already anatomically segregated main and vomeronasal olfactory systems, but at a cellular and molecular level the systems are not yet completely separated. We have recently shown that some V2Rs are specifically expressed in the main olfactory epithelium (MOE) of larval Xenopus laevis, and that amino acid responses in the lateral epithelium overlap with the v2r-expressing zone. Here we show that the laterally located subpopulation of amino acid-sensitive receptor neurons possess a PLC-mediated signaling cascade, that trpc2 is broadly expressed in the MOE and it participates in amino acid signaling. This is the first evidence for a widespread PLC-TRPC2 transduction pathway in the MOE of a vertebrate having a vomeronasal organ. Furthermore, the epithelial localization of trpc2-expressing

cells supports our hypothesis that some MOE-specific V2Rs in amphibians may be amino acid receptors. Together, our results emphasize the intermediary state of the amphibian olfactory system and underline its suitability to study the transition of aquatic to terrestrial olfaction in vertebrates. [Supported by DFG SPP 1392 (I.M and S.I.K) and DFG CNMPB (I.M).]

## #P075 Vibrational vs. physicochemical descriptors for olfactory receptor response prediction

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Responses of olfactory receptors (ORs) can be predicted by applying machine learning methods on a multivariate encoding of an odorant's chemical structure [1]. Many studies used physicochemical descriptors that encode features of the molecular graph. Here, we explore features derived from a molecule's vibrational spectrum, the EVA descriptor [2]. We assessed the performance of support vector regression to predict the gradual response of Drosophila ORs as annotated in the DoOR database [3] and tested the predictions in vivo. We compared a 27-dimensional variant of the EVA descriptor against a set of 32 physicochemical features proposed as an odor metric [4] ("HADDAD"). EVA and HADDAD predicted odor-OR interactions with similar accuracy, indicating that EVA is well suited to represent odor space. However, this observation doesn't argue for the vibration theory of olfaction [5]. Since an odorant's vibrational spectrum is determined by its molecular structure, EVA provides similar information as physicochemical descriptors.Funded by DFG SPP 1392.[1] Schmuker et al., Chem Cent J 2007[2] Ferguson et al., J Comput-Aided Mol Des 1997[3] Galizia et al., Chem Senses 2010[4] Haddad et al., Nat Methods 2008[5] Turin, Chem Senses 1996

## #P076 Elemental or configural perception of an odour mixture induces differences in neural activation in the newborn rabbit

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Mammals are exposed lifelong to a huge number of complex odours. To better understand how the brain processes odorants in mixtures we used the rabbit pup as a model. Pups display sucking behaviour in response to the mammary pheromone (MP). The MP also promotes associative conditioning and rapid acquisition of any novel odorant paired with it. Our previous results showed that after conditioning to ethyl maltol (odorant A), pups do not respond to odorant B (ethyl isobutyrate) or to the AB mixture (70/30 ratio), but they respond to A and to the A'B' mixture (32/68 ratio). These results suggest configural perception of AB and elemental perception of A'B'. Our hypothesis was that levels of neuronal brain activation should differ depending on the mode of perception. Ongoing analyses of c-Fos immunodetection show a stronger activation in the anterior and posterior piriform cortex, tenia tecta and medial amygdala in pups exposed to A'B' compared to pups exposed to AB. Analyses of the olfactory bulb are in progress. These first results suggest that the same odorants may induce different brain activation, and consecutively different behavioural responsiveness, depending on whether their mixtures are perceived elementally or configurally.

## #P077 Prognostic value of nasal thallium-201 transport to olfactory bulb in patients with olfactory disorders

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Objectives: To show the prognostic value of nasally administered thallium-201 transport to olfactory bulb in patients with olfactory disorders. Methods: 20 patients with olfactory disorders were enrolled in the study (10 women and 10 men; 23–71 years old). The causes of olfactory dysfunction in the patients were head trauma (n = 5), upper respiratory tract infection (n = 6), chronic rhinosinusitis (n = 6), and idiopathic (n = 3). Thallium-201 was administered unilaterally to the olfactory cleft, and SPECT-CT was conducted 24 h later. Separate MRI images were merged with the SPECT images. The improvement was judged according to the criteria of the Japanese Rhinologic Society. Log-rank tests and Cox-proportion hazard tests were performed for the

statistical analysis. Results: The period to the improvement was significantly shorter in the patients with high nasal thallium-201 transport to the olfactory bulb than in the patients with low thallium-201 transport to the olfactory bulb. The prognostic value of nasal thallium-201 transport to the olfactory bulb was also significant in the multivariable analysis. Conclusions: Nasal thallium-201 transport to olfactory bulb was useful to predict the prognosis of olfactory-impaired patients.

## #P078 Chemosensory basis for feeding behaviour differences in the larvae of two closely related butterfly species: Papilio hospiton, a specialist and Papilio machaon, a generalist

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The spike activity of the medial (M) and lateral (L) maxillary styloconic taste sensilla of two genetically close species of lepidopterous larvae (Papilio machaon and Papilio hospiton, oligophagous and monophagous, respectively), was recorded after stimulation with deterrent (NaCl at high concentration and nicotine) and phagostimulant compounds (sucrose, glucose, fructose and myo-inositol), with the aim of comparing their response patterns in the light of their different feeding choices. The results show that the spike responses of the maxillary sensilla of two species are statistically different. The neurons activated by phagostimulant or deterrent stimuli are the same in both species and for both sensilla, but P. hospiton shows a higher sensitivity with respect to P. machaon. Finally, the activation of neurons responding to phagostimulants in P. machaon inhibits the neurons responding to deterrents even at low concentrations but, in P. hospiton, only at high concentrations. In conclusion, the higher sensitivity of P. hospiton with respect to P. machaon can justify the differences in feeding behaviour.

## #P079 Enhancement of conditioned taste aversion after long-term intracranial injection of oxytocin in mice

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Oxytocin (OT) contributes to emotional learning, such as fear conditioning, and several social behaviors, including maternal or aggressive behavior. To examine the effect of OT on conditioned taste aversion (CTA), which is a type of emotional learning, we analyzed the degree of acquisition and extinction of CTA memory after a 1-shot (one day) or long-term (20 days) OT injection into the lateral ventricle of mice. Both acquisition and retention of CTA memory were enhanced by long-term OT injection but not by 1-shot OT injection. These results suggest that long-term intracranial exposure to OT enhances acquisition process of CTA memory, resulting in the reinforcement of retention process.

## #P080 Ancestral amphibian V2Rs are expressed in the main olfactory epithelium

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Mammalian olfactory receptor families are segregated into different olfactory organs, with v2r genes expressed in a basal layer of the vomeronasal epithelium. We report here that, the v2r gene family of the amphibian Xenopus laevis is expressed in the MOE as well as the VNO. Interestingly, late diverging v2r genes are expressed exclusively in the VNO, whereas 'ancestral' v2r genes including the single member of v2r family C are restricted to the MOE. Moreover, within the MOE v2r genes are expressed in a basal zone, partially overlapping, but clearly distinct from an apical zone of omp2- and odorant receptor-expressing cells. These zones are also apparent in the spatial distribution of odor responses, enabling a tentative assignment of odor responses to olfactory receptor gene families. Responses to alcohols, aldehydes and ketones show an apical localization, consistent with being mediated by odorant receptors, whereas amino acid responses overlap extensively with the basal v2r-expressing zone. The unique bimodal v2r expression pattern in main and accessory olfactory system of amphibians presents an excellent opportunity to study the transition of v2r gene expression during evolution of higher vertebrates.

## #P081 Olfactory trigeminal interaction

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The sense of smell combines input from the olfactory and the trigeminal system. The latter not only conveys somatosensory information, but also contributes to odour detection. It is suggested that the olfactory and the trigeminal system interact and modulate each other´s sensory properties. An interaction may take place on the peripheral and central level of information processing. Upon stimulation, trigeminal fibers innervating the

olfactory epithelium (OE) and the olfactory bulb (OB) release peptides like Substance P (SP). In calcium imaging experiments in slices of mouse OE, SP elicited a calcium response in a population of cells. Immunohistochemical data indicate co-expression of the SP receptor NK1R, the IP3 receptor, and PLC in a population of cells that differ from regular olfactory neurons. The function of these cells is still elusive.In the OB, trigeminal innervation is most prominent in ventral glomeruli. It was suggested that dorsal regions of the OB process innate odour responses, while learned responses are processed more ventrally. We observed an increase in trigeminal innervation during postnatal development, indicating that trigeminal innervation may be involved in a developmental process, e.g. olfactory learning

## #P082 Effects of bitter compounds on TRPM5 and IP3 receptors

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TRPM5 is a Ca2+-activated non-selective cation channel involved in the transduction of sweet, bitter and umami tastes. We have previously shown that TRPM5 is a key locus for the modulation of taste perception by temperature changes and by quinine and quinidine, two bitter compounds that suppress gustatory responses. Here we determined whether other bitter compounds known to modulate taste perception also affect TRPM5. We found that TRPM5 currents are inhibited by nicotine, with an IC50 of 1.3 mM at -50 mV. Nicotine-induced inhibition results from a reduction of the maximal whole-cell conductance and an acceleration of channel closure that leads to a negative shift of the activation curve. TRPM5 currents were not affected by nicotine's metabolite cotinine, the bitter xanthines caffeine, theobromine and theophilline, or by the intensive sweetener saccharin. In contrast to its inhibitory effect on TRPM5, nicotine did not inhibit IP3 receptors, which are other essential elements of the sweet taste transduction pathway. Nicotine-induced inhibition of TRPM5 may contribute to the inhibitory effect of nicotine on gustatory responses and implies the existence of a TRPM5-independent pathway for the detection of nicotine bitterness.

## #P083 Interaction of lipopolysaccharides with plasma membranes as possible trigger of TRP channel activation

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Lipopolysaccharides and especially its lipid part, Lipid A, are important for the induction of inflammatory responses leading to bacteria clearance. Classical signalling pathways are relatively slow, involving TLR4 activation, recruitment of immune cells, etc. It has been recently found that TRPA1 can sense LPS in nociceptive neurons, independent of the TLR4 pathway. Notably, conical Lipid A from E. coli strongly activates TRPA1, unlike cylindrical Lipid A from S. minnesota. Considering the hydrophobic nature of LPS and the role of TRPA1 channel in mechanosensation we hypothesized that TRPA1 senses the disturbances induced by insertion of LPS in the plasma membrane. By measuring membrane fluidity using membrane-intercalating fluorescence probes we found that E. coli LPS, but not S. minnesota LPS, causes a membrane phase shift towards gel-like state. This suggests that Lipid A structural differences are important in LPS ability to induce mechanical alterations in the membrane. Our results suggest for a novel mechanism of LPS recognition, whereby Lipid A insertion in the plasma membrane causes rigidness, which in turn may induce TRPA1 activation. Thus, TRPA1 seems to act as a mechanosensor to accomplish a key chemosensory function.

## #P084 Mustard oil sensitizes the capsaicin receptor TRPV1 to extracellular acidosis and heat

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Allyl isothiocyanate (AITC, aka mustard oil) is a powerful irritant produced by Brassica plants as a defensive trait against herbivores and confers pungency to mustard and wasabi. AITC is widely used experimentally to induce acute pain and neurogenic inflammation. Here we tested whether AITC sensitizes TRPV1 for activation by extracellular acidosis and heat. Patch-clamp experiments in TRPV1-expressing HEK293T cells revealed that AITC enhances the responses to low pH and heat. These results were confirmed with intracellular Ca2+ imaging experiments in the same cells and in dorsal root ganglion (DRG) neurons isolated from Trpa1 knockout (KO) mice. The responses to low pH and heat in the presence of AITC were strongly reduced by the TRPV1 inhibitor capsazepine and nearly absent in DRG neurons isolated from Trpa1/Trpv1 KO mice. The mechanism of cross sensitization between AITC, low pH and heat occurs via the induction of additive shifts of the voltage dependence of channel activation. These findings indicate that TRPV1 is a locus for cross sensitization between AITC, acidosis and heat in nociceptive neurons and help understanding the molecular bases underlying the role of this channel as mediator of the algesic properties of AITC

## #P085 Functional analysis of nematode GPCRS In yeast

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The yeast Saccharomyces cerevisiae has been used extensively for ligand screening of human G-protein coupled receptors, due to its ease of genetic manipulation, low cost, rapid growth, and eukaryotic secretory pathway. Although the Caenorhabditis elegans genome was sequenced 13 years ago and encodes over 1,000 GPCRs, of which several hundred are believed to respond to volatile organic ligands, only one of these receptors, ODR-10, has been linked to a volatile ligand, diacetyl. ODR-3 is a G-protein α subunit believed to be involved in odorant detection and activated by ODR-10. Here we report the functional coupling of ODR-10 to the yeast pheromone signalling pathway using the yeast - C. elegans chimaeric Gα subunit (GPA-1:ODR-3). We also report the tailoring of a yeast strain for the analysis of C. elegans chemoreceptor function. In this study, a yeast gpa1 ste2 sst2 far1 quadruple mutant strain was constructed to efficiently couple nematode olfactory receptors with the yeast signalling pathway. We used LacZ reporter, to verify activation of the signal transduction pathway by ligand activated GPCR interactions. With this heterologously engineered yeast system, we aim to accelerate the de-orphaning of C. elegans GPCR proteins.

## #P086 Olfactory imprinting in zebrafish: Behavioral and genetic mechanisms

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K. Tietje, C. Hinz, G. GerlachImprinting is a learning process during early development that is limited to a short period of time and leads to irreversible changes in behavior.Based on behavioral experiments we showed that zebrafish imprint on olfactory cues of kin at day 6 of development. Larvae use the learned cues to identify kin. Imprinting does not occur when larvae experience cues of non-kin, suggesting a genetic predisposition for kin odor. We show that MHC genes are relevant for imprinting and kin recognition. Larvae which grew up with MHC II identical kin developed kin discrimination ability, whereas olfactory imprinting did not proceed when larvae were exposed to non-kin odor.To evaluate whether MHC peptides influence olfactory imprinting we exposed larvae to artificial MHC peptides. One fish line showed an olfactory preference for peptides and for kin. We conclude that MHC peptides evoke imprinting in zebrafish.Based on these results we address the question whether imprinting leads to structural changes in the nasal epithelium. We will study differences in olfactory receptor gene expression before, during and after olfactory imprinting. We thank Deutsche Forschungsgesellschaft SPP 1392 for funding.

## #P087 Brain activation pattern during perception of PROP bitter taste using functional magnetic resonance imaging (fMRI)

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Since many toxic substances are bitter, the genetic ability to recognize them confers significant survival advantages to humans. In PROP super-tasters, bitter taste inputs represent important warning signals, which are absent or less effective in non-tasters. Several investigations have attempted to elucidate the molecular basis and the genetic and psychophysical features of this trait. In this preliminary fMRI study (GE-SIGNA 1.5 Tesla) we describe the different brain activations based on the individual taste perception of PROP. We examined one super-taster subject and one non-taster previously classified by their PROP responses. PROP was delivered to the subjects by filter paper disks impregnated with the compound. No statistical significant differences of activity were detected between the two participants before stimulation, while during the administration of PROP, significant differences in cortical activation were detected in the dorsolateral prefrontal region. These preliminary data suggest that the dorsolateral prefrontal cortex is involved in the conscious perception of PROP, which gives rise to a pattern of activity consistent with the different individual ability to taste this compound.

## #P088 The study of Odorant binding proteins (OBPs) as a sensitive layer for label free biosensors for vapour and liquid applications

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OBPs are small and soluble proteins, which are expressed in high concentration in the olfactory structures of many species. The attractive features of OBPs, such as the resistance to degradation at high temperatures and their ability to bind odorants and pheromones reversibly make them a suitable

recognition element for chemical sensors.We are developing biosensors based on olfactory proteins for monitoring the quality of perishable food. We investigated the possibility to use OBPs as a sensitive layer in order to realise a sensor for vapour and liquid applications. We expressed OBPs from a range of different species of mammals and insects. OBPs were immobilised on the gold surfaces of quartz crystal microbalances and capacitive electrodes utilising self assembled monolayers of alkanethiols. The sensors were tested in vapour and liquid toward analytes related to the quality of food. Different OBPs displayed varying degrees of selectivity to individual target analytes, allowing the design of arrays of OBPs that can discriminate single organic compounds. The sensors developed showed a good sensitivity responding to analytes in the order of parts per billion in the vapour phase and nanomolar concentrations in the liquid phase.

## #P089 L-Phenylalanine affects secretion of the hunger hormone, ghrelin, via the calcium sensing receptor

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Ghrelin, a 28 amino acid octanoylated peptide produced in the stomach, stimulates food intake. There is no consensus on the effect of dietary protein on the postprandial decrease in plasma ghrelin levels. This study aimed to investigate how amino acids are sensed by the ghrelin cell. Methods. The effect of L-Phe, in the presence or absence of an antagonist, on ghrelin secretion was studied in vitro in the ghrelinoma cell line, MGN3-1. In vivo, mice were injected intravenously with 100mM L-Phe. Octanoyl ghrelin levels were determined by radioimmunoassay.Results. The ghrelinoma cell line expresses the CaSR, TAS1R1-TAS1R3 and GPRC6A. L-Phe (10mM) increased ghrelin secretion with 65% in vitro. This effect was mimicked by the CaSR agonist, cinacalcet, and blocked by the CaSR antagonist, calhex-231. Intravenous injection of L-Phe in mice decreased the ghrelin content in the stomach, without affecting plasma ghrelin levels. Ghrelin mRNA expression levels were increased in the stomach. Conclusion. L-Phe increases octanoyl ghrelin secretion in the MGN3-1 cell line via the CaSR. In vivo studies suggested that L-Phe is sensed by the CaSR on the ghrelin cell via blood borne direction.

## #P090 Gender specific responses to predator chemical cues in the house mouse: the effects of early olfactory experience

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Finding of universal carnivore signal (Ferrero et al., 2011) does not explain why only odors from natural predators produce profound effects on the behavior and specifically on the neuroendocrine system of prey. It suggests the ability of prey to distinguish predators species on a chemosensory basis. Domestic cat is the most specialized predator to the house mouse. Long history of coexistence in the same environments led to the development of mutual adaptations at the genetic level. Felinine is a species specific compound found in the urine of domestic cats. We compared effects of intact cat urine and felinine/derivatives in a number of tests; analyzed effects of short term and long-term exposures in male and female mice. Females responded to cat urine/felinine with block of pregnancy in 30–70% of cases (depending on the season) and skewed sex ratio in favor of males. Acute exposures caused significant corticosterone response (CRTr) in females but not in males. Long lasting exposure to felinine/cat urine caused significant CRTr in both sexes. Early ontogenetic exposures affected olfactory thresholds to cat urine/felinine and behavioral patterns but not CRTr suggesting an innate nature of CRTr mice to the odor of domestic cat.

## #P091 Opening of an alternative ion permeation pathway in a nociceptor TRP channel

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Sensory neurons detect chemical stimuli through projections in the skin and mucosa, where several transient receptor potential (TRP) channels act as primary chemosensors. Functional TRP channels are tetramers, and it is generally accepted that binding of distinct chemical ligands causes the opening of a single central cation-conducting pore. Contrary to this view, we here provide evidence for a second cation permeation pathway in the TRP channel TRPM3, which can be gated by combined application of endogenous neurosteroids and exogenous chemicals such as clotrimazole or related drugs. This alternative pathway is preserved following desensitization, blockade, mutagenesis and chemical modification of the central TRPM3 pore. Massive influx of Na+ via this alternative pathway enhances excitation of sensory neurons and thereby strongly exacerbates TRPM3-dependent pain. Our findings demonstrate that a single sensory TRP channel can encompass two distinct ionotropic chemoreceptors,

which may have important ramifications for TRP channel function and pharmacology.

## #P092 Molecular mechanisms of olfactory detection in Spodoptera littoralis: Deorphanization of odorant receptors via the Drosophila empty neuron system

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The olfactory sense determines vital steps in insect behaviour, including mate and food seeking, oviposition site selection and predator/parasitoid avoidance. We have established the noctuid moth, Spodoptera littoralis, as a model for investigation of noctuid olfaction and chemical ecology. At the molecular level, insect interactions with the olfactory environment are mediated by odorant receptor (OR) proteins, which are functionally expressed in odorant receptor neurons within olfactory appendages, primarily the antennae.Our aim is to deorphanize the S. littoralis OR genes. Individual ORs are expressed in the Empty Neuron system of the fruit fly, Drosophila melanogaster, and their response profiles are identified by means of single sensillum electrophysiological recordings. We have also utilized gas chromatography analysis of plant headspace extracts, coupled to single sensillum recordings to analyze the tuning of specific ORs to components of ecologically relevant complex odor blends. Preliminary data demonstrate successful adaptation of these methods to the deorphanisation of S. littoralis ORs. These results represent an important step in understanding the molecular mechanisms of olfactory mediated behaviours in S.littoralis.

## #P093 Analyzing the coding scheme in the olfactory bulb with NMF

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Olfactory receptors identify odorants according to the chemical features of the odor molecule, called epitopes. As receptor neurons of the same type converge to the same glomerulus in the olfactory bulb, it is assumed that information coding in the bulb is organized by an epitope map. By using the 2-DG-uptake-technique on rat olfactory bulbs, these odorant specific activity patterns can be illustrated. We used the non-negative matrix factorization (NMF) on 143 distinct uptake-images of the rat olfactory bulb in order to learn about the underlying coding scheme. NMF is designed for revealing inherent structures and similarities in the data and creates a decomposition in parts which display common features of the data. NMF results into up to 9 stable regions (modules), which show high analogy to cluster regions found in other studies. We will show how these modules correlate with well-known odorant characteristics, such as functional groups, molecule size or structural features. This further suggests to understand the modules as single characters that form the odorant coding alphabet. Finally, a better understanding of this code could be the beginning for a deeper understanding of the resulting odor space.

## #P094 Profiling of OR genes expression in the human olfactory epithelium

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Olfactory recognition is mediated by a large repertoire of olfactory receptors. The human genome contains 851 OR loci. More than 50% of the loci are annotated as nonfunctional due to frame-disrupting mutations. Therefore, profiling of OR genes expression in the olfactory epithelium provides an opportunity to select frequently expressed and potentially functional ORs for large deorphanization campaigns. An AB TaqMan® Low Density Array containing probes for 356 predicted OR loci was designed to investigate the expression of the chemosensory receptor genes expression in olfactory epithelium tissues from 15 individuals. Total RNA isolation, DNase treatment, RNA integrity evaluation and reverse transcription were performed for these samples. Then 384 targeted genes were analyzed using the same RT-qPCR platform. The expression of 246 human OR genes was observed in the selected olfactory epithelia, among which 116 were expressed in all individuals. No relation between the number of OR genes expressed and age or sex of individuals was observed. Most of the ORs deorphanized at ChemCom or described in the literature were found in the expressed set.

## #P095 Profiling of TAAR gene expression in human olfactory epithelium and in vitro characterization of the hTAAR1 and hTAAR5

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Profiling of trace amine-associated receptor (TAAR) genes expression was performed in 15 human olfactory epithelia from autopsy. After total RNA extraction, DNase treatment, RNA integrity evaluation and reverse transcription, the expression of the 6 targeted TAAR genes and reference genes for normalization was analyzed using a real-time reverse transcription PCR platform. Upon normalization and quantification, we observed that hTAAR5 was expressed at a relatively high level in most samples (14/15) whereas hTAAR1 was detected at trace level in most samples (12/15). The expression of hTAAR2, hTAAR6, hTAAR8, and hTAAR9 -at a trace level- was noted in a limited number of samples. An agonist screening of a 461 odorant compound library was performed on recombinant human TAARs expressed in HEK293T-hRTP1S/hRTP2 cells using a CRE-luciferase reporter assay. A series of new linear and cyclic alkylamines were identified as potent activators of TAAR1. Most of them are characterized by an unpleasant fishy or ammoniacal odor shared by many alkylamines. These findings suggest that hTAAR1, as well as hTAAR5, may specifically function as chemosensory receptors in human.

## #P096 Functional analysis of umami receptors in the chorda tympani and the glossopharyngeal nerves from mGluR4 knockout mice

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Among five potential umami receptors (T1R1+T1R3, taste-mGluR1, taste-mGluR4, full-length mGluR1 and full-length mGluR4), functional evidences of T1R1 + T1R3 have been accumulated by many reports. However, functional in vivo evidences of mGluR4 are not enough so far. Here, we examined taste responses of the chorda tympani (CT) and the glossopharyngeal (GL) nerves from wild-type mice and mice genetically lacking mGluR4. Responses to MPG with and without IMP were significantly diminished but not abolished both in the CT and the GL of mGluR4-KO mice. Further we examined influence of antagonists for T1R1+T1R3, group I mGluRs, group III mGluRs, NMDA, AMPA and kainic acid receptors on the residual responses of mGluR4-KO mice. Consequently, an antagonist for group I mGluRs, AIDA, suppressed MPG responses in the CT and the GL, and an antagonist for NMDA suppressed MPG response in the GL in mGluR4-KO mice. These data suggests that taste cells that are innervated by the CT possess mGluR1, mGluR4 and T1R1 + T1R3, and taste cells that are innervated by the GL possess mGluR1, mGluR4 and NMDA receptors.

## #P097 Odorant binding protein activity prediction: the pathway for in silico screening

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Odorant binding proteins (OBP) can be explored for ligand binding affinity via in silico screening. However the most common docking software and online services available present different problems in terms of results reliability and prediction potential. Recent technical advances increase the accuracy of these systems making them suitable for screening of OBP/ligands interactions.The aim of this study is to compare the results obtained from in silico simulated and classical chemical binding assays. An expressed and purified recombinant Major Urinary Protein (MUP) and its digital crystal model were tested for binding capability with different molecules including odorants.The results from digital docking were processed, analyzed and compared to those from classical binding experiments. Our data suggest that the digital simulation can provide useful information for screening of a large number of molecules.

## # Transcriptome analysis of individual Tas1r3-expressing taste receptor cells

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Many genes important for sweet taste cell function have been identified, including Tas1r receptors, gustducin, Trpm5, and glucose transporters and K-ATP channels potentially involved in sugar detection. However, much remains unknown about these cells. We have used whole genome transcriptomics to identify all genes selectively expressed in individual Tas1r3+ mouse taste cells. We standardized methods for isolating single Tas1r3+ taste cells and linearly amplifying their mRNA populations. Transcriptome profiling was done by Illumina deep sequencing. Statistical analysis of each taste cell's transcriptome was done for 15 Tas1r3+ cells, then compared to individual transcriptomes from 25 type III cells and five Lgr5+ stem/progenitor cells. These cell types form multiple distinct groups with clearest distinctions between the type II and type III cells. Many marker genes specific to each taste cell type are differentially expressed

several hundred-fold to several thousand-fold, confirming our method's reliability. We are using this method to identify signaling pathways and transcription factors unique to each type of mature taste cell. This data-mining procedure has the potential to lead to the discovery of hitherto unknown receptors.

## # Fabricate A Novel Biosensor with 3-D Print Technology

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With the increasing threat of environment toxicants including biological and chemical warfare agents, fabricating innovative biomimetic system to detect these harmful and dangerous agents is critically important. Various approaches to develop artificial odorants detectors have been reported. However, it has been difficult to develop chemical sensor or biosensor that possesses all natural noses sensing properties. Insect has a highly sensitive and selective discrimination capacity for odorants in the ambience. Its olfactory signaling transduction is ligand-gated ion channels, independently of G-protein signaling, whose olfactory system is much simpler than mammalian's. Thus, insect cells represent attractive tools for realizing highly selective and sensitive biosensors. In the current study, we screened for olfactory receptors that could report the presence of the odorant TNT from insect species and co-expressed these olfactory receptors genes and a companion receptor in Xenopus oocytes or HEK cells. Integration of novel 3-D print technology with molecular engineer technology fabricates biosensors will be a feasible direction to detect harmful and dangerous odorants.

## # Biosensors using Drosophila olfactory receptors for volatile chemicals detection

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In insects such as Drosophila melanogaster, highly sensitive olfaction has been evolved to search for food, find mates, and avoid predators. The Drosophila ORs can be directly gated by odorants, and can function without the co-expression of downstream signaling molecules, which is different with the complex olfactory signaling in mammalian. Therefore, Drosophila ORs hold great promise as detectors in a biosensor for detection of volatile chemicals. Several important OR genes in Drosophila, which respond to explosive vapor or related precursors such as PETN and 2-ehtyl-hexanol, have been reported. The present work screened and selected OR genes which are sensitive to explosive vapor and related precursors by calcium imaging and voltage clamping techniques. Furthermore, a biosensor which co-expresses these OR genes and a companion receptor Or83b in living cells is being constructed, in order to classify unknown volatile chemicals and detect specific types of illicit substances. This study provides an alternative way for the construction of odorant sensors, and would promote the development and application of biosensors in a range of applications such as security and environmental monitoring.