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ABSTRACTS

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Organizers: Stefan H. Fuss and Çağri Çevrim

KEYNOTE LECTURES

K1 - Deconstructing Smell.

Linda B. Buck

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The mammalian olfactory system possesses immense discriminatory power. It can generate diverse odor perceptions to a vast array of volatile odorants and stimulate instinctive behaviors and hormonal changes to conspecific and interspecific social cues. Our work has focused on two questions. First, how do mammals detect so many different chemicals. And second, how does the nervous system translate those chemicals into diverse perceptions and innate responses. Using a combination of molecular, cellular, and genetic approaches, we have identified four different families of chemosensory receptors expressed by sensory neurons in the olfactory epithelium and vomeronasal organ and interrogated how signals from different receptors are organized and used to encode chemical identities in these peripheral sense organs and in the main olfactory bulb. Our recent studies indicate that neurons expressing odorant receptors (ORs) and TAARs belong to different lineages of nasal sensory neurons and that distinct nuclear locations of these receptors may underlie their segregated expression. We are currently using viral tracers and pharmacogenetic methods to gain insight into the neural circuits underlying instinctive responses to social cues.

K2 - Taste Cells of the Gut and Endocrine Cells of the Tongue.

Robert F. Margolskee

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We have found that many of the receptors and downstream signalling elements involved in taste detection and transduction are expressed also in intestinal hormone producing (endocrine) cells where they underlie key chemosensory functions of the gut. In one example of gastrointestinal chemosensation it is known that glucose given orally, but not systemically, induces secretion of the "incretin" hormone GLP-1 (glucagon like peptide-1), which in turn regulates insulin secretion and glucose homeostasis. We have found that intestinal endocrine cells express sweet taste receptors, the taste G-protein gustducin, and several other taste transduction elements. Knockout mice lacking gustducin or the sweet taste receptor subunit T1R3 have deficiencies in secretion of GLP-1 and in the regulation of plasma levels of insulin and glucose. In another example of gastrointestinal chemosensation we have found that endocrine cells of the pancreas express multiple taste proteins that are involved in regulating insulin release. Furthermore, taste cells of the oral cavity express GLP-1, other "gut" hormones and the insulin receptor. Recently, we identified intestinal-type glucose transporters and pancreatic-type ATP-gated K+ channels (K-ATP metabolic sensors) as being present in taste cells and potentially functioning in the detection of the sweet taste of sugars. Most recently, we have found that the intestinal brush border disaccharidases maltase glucoamylase and sucrase-isomaltase are expressed in T1R3+ taste cells. Further, we found that two disaccharidase inhibitors significantly reduced gustatory nerve responses to sucrose and maltose, but not to the monosaccharides glucose and fructose or the noncaloric sweeteners. We propose that these enzymes act in concert with salivary amylase to generate free glucose from sucrose, maltose and starch that can activate the T1R3-independent sugar detection pathway. In sum our studies point out similarities in gustation and gut chemosensation and indicate the importance of "taste cells of the gut" and "endocrine cells of the tongue" in coordinating the body's hormone responses to regulate glucose homeostasis.

K3 - Olfactory Circuits: From Receptors to Behavior.

Hitoshi Sakano

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In the mouse olfactory system, individual glomeruli in the olfactory bulb (OB) represent a single type of odorant receptor (OR). A predator odor, TMT (trimethyl-thiazoline), activates multiple glomeruli in both the dorsal and ventral regions in the OB. However, a limited number of glomeruli localizing to the posterior DII region (J domain) are responsible for

inducing innate fear toward TMT. It remains undetermined whether an individual glomerulus is functionally specialized for a particular innate response, or if a pattern of activated glomeruli is recognized similar to odor discrimination. To address these questions, we generated the knockin (KI) mouse in which a TMT-responsive receptor, ORTMT, is expressed with channelrhodopsin wide-receiver, ChRWR. Photo-activated projection neurons connecting to the ORTMT glomerulus were TMT-responsive. To our surprise, photo-stimulation of the single ORTMT glomerulus induced fear in the KI mouse, demonstrating immobility response and holding whisker movement. Immuno-histochemistry revealed that TMT-responsive regions in the olfactory cortex were activated by photo-illumination in the KI mice. We also analyzed the knockout (KO) mice for ORTMT. In the KO, fear responses to TMT were lowered, but not entirely abolished. This is likely due to the compensatory function of other TMT-responsive glomeruli remaining in the posterior DII region. These results indicate that stimulation of a single glomerulus in the J domain of the OB is sufficient to elicit fear responses in the mouse.

PLENARY SYMPOSIUM I: NEURAL CIRCUITS: FROM RECEPTORS TO BEHAVIOR.

S1 - Molecular and neuronal components of the aversive response to cadaverine in zebrafish.

Sigrun Korsching

University at Cologne, Institute of Genetics, Biocenter, Cologne, Germany

Carrion smell triggers distinct innate behaviors in many species including humans. This smell is mainly caused by the aliphatic diamines putrescine and cadaverine. Depending on the species, these diamines may serve as avoidance cues, feeding attractants, oviposition attractants, or social cues. We have identified a zebrafish olfactory receptor from the TAAR family, TAAR13c, as a sensitive and specific cadaverine receptor and show specific activation of TAAR13c-expressing olfactory sensory neurons by cadaverine. Within the olfactory bulb a single glomerulus is activated at the lowest concentrations of cadaverine. We report that zebrafish avoid a cadaverine source, corresponding to the strongly aversive reaction of humans to cadaverine. These results provide a basis for studying neural circuits connecting sensation, perception, and innate behavior in a vertebrate model system.

S2 - Olfactory receptors, circuits and behaviors in zebrafish.

Yoshihiro Yoshihara

RIKEN Brain Science Institute, Wako, Japan

Many olfactory cues pervade the aquatic environment of fish and induce various behaviors important for their individual survival and species preservation, such as searching foods, escaping from danger, and finding potential mates. The zebrafish has become one of the most useful model organisms in neurobiology. In addition to its general advantageous properties (external fertilization, rapid development, transparency of embryos, etc.), the zebrafish is amenable to various genetic engineering technologies including transgenesis, mutagenesis, gene knockout, and transposon-mediated gene transfer. The zebrafish genome harbors ~300 olfactory receptor genes consisting of ~150 ORs, 6 V1Rs, ~50 V2Rs, and ~100 Taars. "One neuron-one receptor rule" and "glomerular convergence of like axons" are essentially preserved in the zebrafish. A functional odor map is developed on the glomerular array of the olfactory bulb, based on chemical structures of odorants and pheromones: e.g., amino acids in the lateral glomerular cluster, bile acids in the dorsal glomerular cluster, and prostaglandin F2a in the ventromedial glomeruli. The mitral cells project axons to the four major target areas in the forebrain: the posterior zone of dorsal telencephalic area (Dp), the ventral nucleus of ventral telencephalic area (Vv), the right habenula (rHb), and the posterior tuberculum (PT). In this talk, I will introduce our recent findings on the neural circuit mechanism underlying the olfactory alarm response in zebrafish, which is induced by putative alarm pheromones (Schreckstoff) released from injured skin of conspecifics.

S3 - Understanding chemosensory processing and decision-making in Drosophila.

Ilona Grunwald Kadow

Max-Planck Institute of Neurobiology, Martinsried, Germany

When interacting with their environment animals constantly have to make decisions. These decisions usually aim at maximizing reward while avoiding negative consequences such as energy costs, pain, or long-term disadvantages. Faced with a choice animals consider and integrate several parameters such as their internal state as well as other external stimuli. Therefore, even innate preferences need to be evaluated in a context-dependent manner and hence, context strongly impinges on behavior. While it is generally accepted, that context influences behavior our knowledge of the neural mechanisms of how internal state and external conditions alter behavioral outcomes is scarce. We are interested in understanding how context influences neural processing and behavior to odors and tastes. To this aim, my lab combines Drosophila behavioral analysis, in vivo functional imaging, optogenetics, and electrophysiology with state of the art genetic methods to understand how context influences neural processing and ultimately behavior. I will present data on the role of the higher brain centres in the insect brain, such as the mushroom body, and how we think they are used to integrate sensory information and internal state to guide instantaneous value-based decision making and behaviour.

S4 - Synaptic and circuit mechanisms for flexible olfactory processing in Drosophila.

Vanessa Ruta

The Rockefeller University, New York, USA

In a complex and dynamic environment, animals must constantly vary their behavior to accommodate changing circumstances and contingencies. Yet, how associative brain centers can flexibly couple a single sensory input to alternative behavioral outputs remains unclear. We are exploiting the relative simplicity of the Drosophila olfactory system to gain insight into the synaptic and circuit mechanisms through which context and experience can modify odor processing. In Drosophila, the mushroom body is a higher brain center that integrates olfactory and contextual signals to generate flexible and adaptive behaviors. Using functional synaptic imaging and electrophysiology, we show that the mushroom body functions like a switchboard in which dopaminergic neuromodulation can reroute the same odor signals to different behavioral circuits depending on the state and experience of the fly. Our data suggest a circuit mechanism for behavioral flexibility in which neuromodulatory networks act with exquisite spatial precision to transform a single sensory input into different patterns of output activity.

S5 - Molecular and neural sensing of social cues.

Yoh Isogai and Catherine Dulac

Howard Hughes Medical Institute, Harvard University, Department of Molecular and Cellular Biology, Cambridge, USA

One of the fascinating problems in neuroscience is to understand the neural circuits underlying the sociality of animals. Social behaviors - stereotyped behaviors that satisfy social needs – are highly conserved. The sensing of social cues that drive social behaviors is a fundamental question that remains unresolved. In many animals, pheromones are essential social cues. The exact identities of pheromones and their receptors have largely remained elusive in many organisms therefore present an outstanding question. In mice, the vomeronasal organ (VNO) is one of several chemosensory organs implicated in pheromone detection. VNO expresses approximately 300 G-protein coupled receptors. However, the roles of a majority of vomeronasal receptors (VRs) in the detection of social cues have remained unclear. Our previous study (Isogai et al. 2011 Nature) linked approximately 90 VRs to socially and physiologically relevant stimuli in mice. This high-throughput screen allowed us to conclude that VRs encode highly specific identity such as gender and species or physiologically relevant information such as stress and reproductive information. We will discuss our recent work to identify the roles of VRs in specific social behaviors. Our study is now starting to reveal a detailed understanding of behavioral specificity of individual VRs.

S6 - Neural circuitry mediating sexual behavior in mice.

Kazushige Touhara

The University of Tokyo, Department of Applied Biological Chemistry, Tokyo, Japan

In mice, a variety of social and sexual behaviors are regulated by chemosignals called odorants or pheromones. The mechanisms underlying odor and pheromone sensing have been revealed at the levels of receptor and the olfactory bulb since the discovery of chemosensory receptors expressed in the olfactory and/or vomeronasal systems. Recently, molecular biology and neuroscience techniques are being used to elucidate a neural circuitry that mediates odorant- or pheromone-induced behavior in the brain. The exocrine gland-secreting peptide (ESP) family provides a perfect system for this purpose because signaling molecules, their corresponding receptors, and output pheromone behaviors are all lined up. In this talk, I describe novel functions that we recently revealed for some of the ESP family, and then I introduce our challenge to reveal a connectome involved in information processing for ESP-mediated specific behaviors in the brain by regulating activities of specific neuronal types as well as by using a virus tracing technique.

PLENARY SYMPOSIUM II: STRUCTURE-FUNCTION RELATIONSHIPS OF TASTE SIGNALING MOLECULES.

S7 - Signal transmission by ligand-gated ion channels.

Horst Vogel

Swiss Federal Institute of Technology Lausanne (EPFL), Lausanne, Switzerland

Neurotransmitter-gated ion channels of the Cys-loop receptor family mediate fast neurotransmission throughout the nervous system. The molecular processes of neurotransmitter binding, subsequent opening of the ion channel and ion permeation remain poorly understood. Here we present recent results of high-resolution X-ray crystallography, single particle imaging, and molecular modeling studies of a mammalian Cys-loop receptor, the mouse serotonin 5-HT3 receptor. We revealed at atomic detail how neurotransmitter binding on the extracellular domain of the 5-HT3 receptor induces sequential conformational transitions in the receptor opening a transmembrane ion channel: Agonist binding first induced distinct conformational fluctuations of particular side chains in the highly conserved ligand binding cage, followed by tilting-twisting movements of the extracellular domain which coupled to the transmembrane TM2 helices to open the hydrophobic gate and forming a continuous transmembrane water pathway. The structural transitions

in the receptor's transmembrane part finally coupled to the intracellular region opening passages for ion release. The details of structural transitions of the 5-HT3 receptor deliver important insights for understanding the operating mechanism of mammalian Cys-loop receptors.

S8 - Interaction between brazzein and monellin, two sweet-tasting proteins and the T1R2/T1R3 sweet taste receptor.

Loic Briand[1], Anni Laffitte[1], Fabrice Neiers[1], Anne Brockhoff[2], and Wolfgang Meyerhof[2]

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Brazzein and monellin are two naturally occurring plant proteins perceived as sweet by humans, which share no sequence or structural similarity. Both of these proteins like all classes of sweet compounds are perceived through the activation of the T1R2/T1R3 heterodimeric sweet taste receptor. T1R2 and T1R3 subunits are members of the small family of class C G-protein coupled receptors (GPCRs). Class C GPCRs possess a large N-terminal domain (NTD) linked to a heptahelical transmembrane domain by a cysteine rich domain (CRD). The NTD of T1R2 (T1R2-NTD) has been shown to contain the primary binding site for most of the sweet ligands including natural sugars, D-amino acids, and artificial sweeteners. While residues in hT1R2-NTD have been shown to be required for the receptor response to monellin, site-directed mutagenesis have demonstrated that CRD of human T1R3 is determinant for receptor activation to brazzein. In contrast, molecular modeling and docking studies have proposed a hypothetical 'wedge model' in which both of these sweet proteins may interact in a multi-point binding mode to an external site of the sweet receptor NTDs. To elucidate the individual contribution of both receptor subunits to brazzein and monellin detection, we recombinantly expressed human T1R2- and T1R3-NTDs in Escherichia coli. T1R2 and T1R3-NTD were tested separately for their ability to interact with sugar and sweeteners using intrinsinc fluorescence. We then measured the interactions of T1R2 and T1R3-NTDs with the two recombinant sweet-tasting proteins, using Bio-Layer Interferometry (BLI). This optical technique analyzes the signal variations in the interference pattern generated from visible light reflected from an optical layer and a biolayer containing the immobilized protein of interest. This recent method is powerful for studying protein-protein interactions and measuring both affinity constants and kinetic parameters. BLI experiments demonstrated that T1R2-NTD binds brazzein and monellin with Kd values in the physiological range. These affinities are in agreement with the capacity of these sweet-tasting proteins to activate T1R2/T1R3 receptor heterologously expressed in HEK cells and with sensory experiments conducted on humans. We will discuss these data in regards to the wedge model, which will be further investigated using site-directed mutagenesis.

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S9 - The human Histamine H1 receptor in complex with doxepin.

Simone Weyand

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G protein–coupled receptors (GPCRs), also known as seven-transmembrane domain receptors, constitute a large protein family of receptors that sense molecules outside the cell and activate inside signal transduction pathways and, ultimately, cellular responses. This class of proteins includes mammalian olfactory receptors and bitter and sweet taste receptors. The high resolution structure determination of these proteins is still very challenging but recent progress has been made and therefore it is now possible to determine their 3 dimensional structure at atomic resolutions. The human Histamine H1 receptor belongs to the same family of membrane proteins and the approach we used to solve its structure by X-ray crystallography will be used to tackle the structures of the sweet and bitter taste receptors. Histamine is an important mediator involved in pathophysiological processes such as allergies and inflammations. Histamine H1 receptor (H1R) antagonists are very effective drugs alleviating the symptoms of allergic reactions. The human Histamine H1 receptor was overexpressed, purified and crystallized in complex with the antihistamine doxepin. The structure shows the H1R complex with doxepin, a first-generation H1R antagonist. Doxepin sits deep in the ligand-binding pocket and directly interacts with Trp428, a highly conserved key residue in G-protein-coupled-receptor activation. This well-conserved pocket with mostly hydrophobic nature contributes to the low selectivity of the first-generation compounds. The pocket is associated with an anion-binding region occupied by a phosphate ion. This study sheds light on the molecular basis of H1R antagonist specificity against H1R. Further the presented work on the Histamine H1 receptor will show how structures give detailed insight into the function of proteins.

S10 - Structure-function of bitter taste receptors.

Maik Behrens, Stefanie Nowak, Antonella Di Pizio, Anat Levit, Masha Y Niv, and Wolfgang Meyerhof

Department of Molecular Genetics, German Institute of Human Nutrition, Potsdam-Rehbruecke, Nuthetal, Germany

The detection of bitter substances in vertebrates is facilitated by an array of bitter taste receptors belonging to the taste 2 receptor family (Tas2r) of G protein-coupled receptors

(GPCRs) expressed in the oral cavity and beyond. First detected in human bitter taste receptors, later confirmed in other vertebrates' Tas2r repertoires, the enormous variability in the sizes of agonist spectra raised considerable interest in the structure-function relationships of these versatile receptors. Of particular interest is the ability of the very broadly tuned receptors since they could contribute disproportionally to vertebrate bitter tasting abilities and conceivably require a special architecture of their ligand binding pockets. At present, such extremely broadly tuned receptors, which are capable to interact with about one-third of all tested bitter substances, have been identified in human, amphibian, and avian species. Detailed structure-function analyses were performed on human "generalist" receptors. These receptors have been investigated by a combination of in silico homology modeling and docking experiments as well as site directed mutagenesis approaches in concert with functional heterologous expression assays in mammalian cell lines. This presentation will focus on structure-function relationships of broadly tuned bitter taste receptors revealing the existence of single agonist binding pockets in receptors with numerous structurally diverse agonists, emphasize the intricate balance between tuning width and strength of agonist interaction, and the characterization of the general binding site architecture. Moreover, the availability of functional data on non-human bitter taste receptors as well as the discovery of naturally occurring bitter receptor antagonists shed light on the evolution of this highly interesting gene family, the evolutionary implications of these findings will be discussed as well. A detailed knowledge about the structural features important for selective agonist interaction of bitter taste receptors as well as a better understanding of the chemical structures required for receptor activation may allow the rational design of agonists and antagonists required for research and bitter masking of e.g. drugs.

S11 - Genuine type II like human bitter taste cells: a smart tool to investigate gain- and loss-of- function of TAS2Rs.

Katja Riedel, Silke Lambing, Andreas Hochheimer, Michael Salomon, Michael Krohn

BRAIN AG, Zwingenberg, Germany

Among basic taste modalities the human bitter taste plays a particularly vital role and helps us to detect thousands of structurally diverse bitter tasting molecules. Recently, BRAIN established the immortalized human taste cell line HTC-8 from lingual taste epithelium. Functional studies on HTC-8 revealed functional expression of a portfolio of 13 different TAS2Rs. HTC-8 can therefore be used to investigate gain- as well as loss-of-function of human bitter receptors. Moreover, in the context of human taste cells, TAS2Rs interaction and oligomerization to detect bitter taste stimuli can be adressed, which was suggested in previous studies using recombinant non-gustatory cells. Genetic engineering of HTC-8 was established via viral transduction of expression cassettes. Gain-of-function by recombinant TAS2R38 overexpression was established to analyze phenylthiocarbamide (PTC) response by the corresponding bitter taste receptor TAS2R38. Fluorescent Ca-imaging experiments revealed that the PTC response was enhanced in HTC-8:TAS2R38 cells compared to the parental cell line and that sensitivity to PTC was increased as indicated by a decrease of the EC50 for PTC, whereas response to control stimuli was not or only slightly affected. Surprisingly, responses to TAS2R38 non-selective bitter compounds like e.g. saccharin were also enhanced, although RT-PCR analysis confirmed that only TAS2R38 expression was increased in HTC-8:TAS2R38 cells. In order to analyze loss-of-function, RNA interference was used to knockdown the saccharin-responsive TAS2R43 and R44 receptors. Fluorescent Ca-imaging experiments revealed that shRNAs targeting the closely related TAS2R43 and TAS2R44 receptors almost abolished response to saccharin in HTC-8 cells. Surprisingly, shRNA treatment also reduced responsiveness to further bitter tastants, whereas responsiveness and sensitivity to other control stimuli was not or only weakly affected. The results provide new insights into TAS2R function and indicate interaction of TAS2Rs to modulate bitter taste responses in human taste cells.

S12 - 5-HT3 receptors are involved in mediating afferent nerve responses from Type III taste cells.

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While ATP serves as a necessary signal molecule for the transmission of all taste qualities to the taste nerves, other neurotransmitters are present in taste cells and could participate in afferent neurotransmission. In particular, Type III taste cells release serotonin (5-HT) in response to sour taste stimuli, but whether 5-HT plays a role in afferent taste signaling is not known. Using transgenic mice expressing GFP from the 5-HT3a promoter, we show that a subset of geniculate ganglion neurons and afferent taste fibers express GFP, and that the GFP expressing nerve fibers appear to selectively contact Type III taste cells, identified by an antibody against 5-HT. Expression of 5-HT3 receptors in the geniculate ganglion was confirmed by RT-PCR and in situ hybridization. To test functionality of the 5-HT3 receptors, we performed Ca2+ imaging of isolated geniculate ganglion neurons. GFP-labeled neurons responded to both ATP (10 uM) and 5-HT (10 uM), and the response to 5-HT was inhibited by the selective 5-HT3 antagonist ondansetron (1 uM). Furthermore, the response to 5-HT was mimicked in the GFP-labeled neurons by the 5-HT3 specific agonist, m-chlorophenylbiguanide (10 uM). In contrast,

unlabeled neurons, presumably those contacting Type II taste cells, responded only to ATP. To test whether signaling via 5-HT3 receptors affects afferent taste responses, we compared chorda tympani nerve responses to a variety of taste stimuli, including several concentrations of citric acid and HCl, in WT and 5-HT3a knockout mice. Responses to both acids were significantly reduced, but not abolished in the KO mice compared to the WT mice. Interestingly, responses to several other tastants, including some of those transduced by Type II cells, were also slightly reduced in the knockout mice. These effects were mimicked in the wildtype mice by i.p. injection of ondansetron, while injection of vehicle was without effect over the same time course. Of note, the effects of the knockout and the antagonist were only observed when the mice were anesthetized with urethane; no significant effects were observed during anesthesia with pentobarbital, likely because pentobarbital inhibits 5-HT3 receptors at anesthetic doses. These data suggest that 5-HT released during stimulation of Type III taste cells activates 5-HT3 receptors on afferent nerve fibers and partially accounts for the afferent nerve response to sour stimuli. Why stimuli that normally activate Type II cells were also reduced in the absence of 5-HT3 is not clear, but may involve signaling between Type II and Type III taste cells.

PLENARY SYMPOSIUM III: HUMAN OLFACTION – NOTHING TO WRITE HOME ABOUT?

S13 - How the nose knows: A hierarchical overview of the neural processing of odors.

Johan Lundström

Karolinska Institutet, Department of Clinical Neuroscience, Division of Psychology, Stockholm, Sweden

The sense of smell (olfaction) is the only receptor system that exists in all biological domains; from single cell organisms to us, humans. The sense of smell is also unique among the senses in respect of its morphology and connectivity. In this talk, I will provide a hierarchical overview, from odor receptor to cortical processing, of how we make sense of odors with a clear focus on the underlying neural mechanisms of odor perception. I will highlight recent clinical findings regarding how a dual conscious and non-conscious processing streams jointly shape our final olfactory percept and how this percept alters our every-day behavior. Throughout the presentation, I will provide a comparative view of similarities and differences between the human and non-human animals.

S14 - Olfactory receptor genes expression in human olfactory mucosa.

Christophe Verbeurgt, Francoise Wilkin, Maxime Tarabichi, Jacques Emile Dumont Sergio, Hassid, and Pierre Chatelain

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Introduction and aim: Olfactory recognition is mediated by a large repertoire of 851 olfactory receptor loci. In spite of a rather accurate genomic characterization, very little is known about the details of the involvement of human olfactory receptor in odorant perception. So far, the responses of 48 human olfactory receptor with one or more odorant molecules have been reported. Therefore, profiling of olfactory receptor genes expression in whole human olfactory mucosa provides an opportunity to select the frequently expressed and potentially functional olfactory receptors in view of systematic deorphanization. Material and methods: An Applied Biosystems TaqMan® Low Density Array containing probes for 356 predicted human olfactory receptor loci was designed to investigate their expression in whole human olfactory mucosa tissues from 26 individuals (13 women and 13 men, with an average of 67 ± 11 years for women and 63 ± 12 years for men). Total RNA isolation, DNase treatment, RNA integrity evaluation and reverse transcription were performed. Then 384 targeted genes (including reference genes) were analyzed using the same real-time polymerase chain reaction platform. Results: The expression of 273 human olfactory receptor genes was observed in the selected whole human olfactory mucosa, among which 90 were expressed in all individuals. A set of 140 human olfactory receptor genes were detected in more than half of the population and a third set composed of 125 human olfactory receptor genes were more rarely detected. Globally, the olfactory receptor genes expression was not associated with age, sex or smoking. Conclusions: There is a substantial difference in the expressed olfactory receptor gene repertoire of each of the individuals. Most of the olfactory receptors deorphanized on the basis of sensitivity to known odorant molecules, which are described in the literature, were found in the expressed set.

S15 - A new measure of odor complexity is also applicable across senses.

Snitz Kobi, Anat Arzi, Merav Jacobson, Lavi Secundo, Kineret Weissler, and Adi Yablonka

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We defined a new measure of odorants based on the amount of variance in answers to questions about them. Our definition is based on the intuition that odorants (and other stimuli) will vary in the amount of variance they invoke. For example, answers to questions about a red square will be more consistent than questions about a Jackson Pollock painting. Likewise in olfaction, some odorants consistently invoke more variance than others. We call such variance-inducing odorants complex or intricate odors. As a consequence of our definition, our measure is also performance based (as opposed to being based on assessment) and can be applied across modalities. We used three olfactory experiments

to confirm that our measure is well defined and robust. In addition a vision experiment showed that our measure can indeed be applied to other senses consistently. Next weset out to test if our measure is correlated with well established measures of sensory processing. We compared our measure of the intricacy of visual stimuli with their masking effectiveness in an Rapid Serial Visual Presentation experiment. The result was that our measure is correlated at $r=0.75$ $p<0.002$ to masking effectiveness. This shows that our measure is not only well defined but also that it could be relevant to many different phenomenon and open to rich interpretation.

S16 - Olfactory bulb proteome dynamics during the progression of sporadic Alzheimer's disease.

Enrique Santamaria

Proteored-ISCIII, Navarrabiomed, Miguel Servet Foundation, Clinical Neuroproteomics Laboratory, Pamplona, Spain

Olfactory dysfunction is an early symptom of many neurodegenerative diseases, including Alzheimer's disease (AD). Although smell impairment is related to deposition of neuropathologic substrates such as hyperphosphorylated tau protein, and β-amyloid in olfactory areas, the molecular mechanisms associated with decreased smell function are not completely understood. To gain new insights into the underlying pathogenic mechanisms in the olfactory system, we have applied mass spectrometry-based quantitative proteomics to probe additional molecular disturbances in postmortem olfactory bulbs (OB) dissected from pathologically confirmed AD cases respect to neurologically intact controls. More than 4,000 proteins have been identified in human OB. Relative proteome abundance measurements have revealed protein interaction networks progressively disturbed across Braak stages suggesting impaired mitochondrial function, an imbalance in the cycling of neurotransmitters, and a disturbance in neuron-neuron adhesion and neurite growth in the OB during AD pathogenesis..

S17 - Blood, Sweat and Tears: Human social chemosignaling.

Noam Sobel

Weizmann Institute of Science, Department of Neurobiology, Rehovot, Israel

Most animals communicate using social chemosignals, namely chemicals emitted by one member of the species, which then produce chemical and behavioral changes in other members of the species. Such communication is prevalent in insects and terrestrial mammals, and mounting evidence implies that it is also common in human behavior, albeit primarily at a subliminal level. Human social chemosignals are responsible for a host of effects ranging from driving menstrual synchrony in women to conveying fear across individuals. Here I will describe our findings on mechanisms of human chemosignaling in both health and disease. Based on these findings I will build a claim that in contrast to common notions, humans are highly olfactory animals.

PLENARY SYMPOSIUM IV: DISSECTION OF VERTEBRATE OLFACTION.

S18 - Dissecting odor quality.

Stuart Firestein

Columbia University, New York, USA

What makes a molecule an odor? There are numerous psychophysical olfactory paradoxes that one might have thought would heave been resolved with the discovery of a large family of odor receptors. How can three distinct chemical families all create the nearly identical perception of musk? How do we distinguish so dependably between small aliphatic molecules differing by only a carbon atom such as heptyl and hexyl acetate. Why are we unable to predict whether a given molecule will have an odor or not? With so many receptors how is it that there are so many molecules that do not have an odor? We propose in this presentation to reconsider the classification schemes we use for odors based on their biological functionality rather than solely on their chemical properties. This follows many of the pharmacological process known as medicinal chemistry. In particular we explore the idea of bioisosterism.

S19 - Dissecting the olfactory nerve.

Charles Greer

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Our recent efforts have been focused on understanding the initial development of olfactory sensory neuron axons and their organization within the olfactory nerve. Odorant receptors (OR) are strongly implicated in coalescence of olfactory sensory neuron (OSN) axons and the formation of olfactory bulb (OB) glomeruli. However, when ORs are first expressed relative to basal cell division and OSN axon extension is unknown. We developed an in vivo fate-mapping strategy that enabled us to follow OSN maturation and axon extension beginning at the time of basal cell division. In parallel, we mapped the molecular development of OSNs beginning at the time of basal cell division, including the onset of OR expression. Our data demonstrate that ORs are first expressed around 4 days following basal cell division, 24 hours after OSN axons have reached the OB. Over the next 6+ days the OSN axons navigate the OB nerve layer and ultimately coalesce in glomeruli. These data provide a new perspective on the role of ORs in homophilic OSN axon adhesion and lead us to propose a new model dividing

axon extension into two phases. Phase I is OR independent and accounts for up to 50% of the time during which axons approach the OB and begin navigating the olfactory nerve layer. Phase II is OR dependent and concludes as OSN axons coalesce in glomeruli.

S20 - Dissecting Innate Odor Aversion.

Frank Zufall

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Innate predator aversion evoked by predator-derived chemostimuli called kairomones offers a strong selective advantage for potential prey animals. It is unclear how chemically-diverse kairomones can elicit similar avoidance behaviors. Using a combination of behavioral analyses and single-cell Ca2+ imaging in wild-type and gene-targeted mice, we show that innate predator-evoked avoidance is driven by parallel, non-redundant processing of volatile and nonvolatile kairomones through the activation of multiple olfactory subsystems including the Grueneberg ganglion, the vomeronasal organ, and chemosensory neurons within the main olfactory epithelium. Perturbation of chemosensory responses in specific subsystems through disruption of genes encoding key sensory transduction proteins abolished avoidance behaviors and/or cellular Ca2+ responses to different predator odors. Stimulation of these different subsystems resulted in the activation of widely distributed target regions in the olfactory bulb, as assessed by c-Fos expression. However, in each case this c-Fos increase was observed within the same subnuclei of the medial amygdala and ventromedial hypothalamus. Thus, the mammalian olfactory system has evolved multiple, parallel mechanisms for kairomone detection that converge in the brain to facilitate a common behavioral response. Our findings provide significant insights into the genetic substrates and circuit logic of predator-driven, innate aversion and may serve as a valuable model for studying instinctive fear and human emotional and panic disorders.

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S21 - Dissecting the genetic and environmental influences on olfactory receptor expression.

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Detection of odorants occurs in the main olfactory epithelium (MOE), which contains olfactory sensory neurons (OSNs) that express olfactory receptors (ORs). These bind the odorants and then transmit an electrical signal to the brain. The mouse nose has over 1,200 different OSN types, each patterned by a different OR gene. High levels of genomic variation have been reported both in the mouse and human OR repertoire. This is thought to contribute to the unique sense of smell each individual has, but the mechanisms responsible are not known. We have devised an RNAseq-based approach to quantify the OSN repertoire of three inbred strains of mice (C57BL/6, CAST/EiJ and 129S5) via their OR gene expression levels. We found that each strain has a unique and reproducible distribution of OSNs in their noses, and found that genomic variation instructs this neuronal variance in cis. Additionally, OR expression in the MOE is susceptible to olfactory experience. Exposure to an enriched olfactory environment results in the differential expression of dozens of OR genes in a reproducible and specific manner. These changes increase with time and are reversible. These data allows, for the first time, to comprehensively explore and dissect the effects of genetic and environmental variation in the regulation of OR expression and OSN repertoire. Together they generate an olfactory sensory system that is individually unique.

S22 - Coding olfaction.

Peter Mombaerts

Max Planck Research Unit for Neurogenetics, Frankfurt, Germany

Chemosensory specificity in the main olfactory system of the mouse relies on the expression of ~1,100 odorant receptor (OR) genes across millions of olfactory sensory neurons (OSNs) in the main olfactory epithelium (MOE), and on the coalescence of OSN axons into ~3,600 glomeruli in the olfactory bulb. A traditional approach for visualizing OSNs and their axons consists of tagging an OR gene genetically with an axonal marker that is cotranslated with the OR by virtue of an internal ribosome entry site (IRES). We have generated full cell counts for 15 gene-targeted strains of the OR-IRES-marker design coexpressing a fluorescent protein. These strains represent 11 targeted OR genes, a 1% sample of the OR gene repertoire. We took an empirical, "count every cell" strategy: we counted all fluorescent cell profiles with a nuclear profile within the cytoplasm, on all serial coronal sections under a confocal microscope - a total of 685,673 cells in 56 mice at postnatal day 21. We then applied a strain-specific Abercrombie correction to these OSN counts, in order to obtain a closer approximation of the true OSN numbers. We find a 17-fold range in the average (corrected) OSN number across these 11 OR genes. In the same series of coronal sections, we then determined the total volume of the glomeruli (TGV) formed by coalescence of the fluorescent axons. We find a strong linear correlation between OSN number and TGV, suggesting that TGV can be used as a surrogate measurement for estimating OSN numbers in these gene-targeted strains.

PLENARY SYMPOSIUM V: OLFACTION IN INSECTS.

S23 - The role of Orco in pheromone transduction of the hawkmoth Manduca sexta.

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Insect odor receptors (ORs) are 7TM receptors with inverse membrane topology. They associate with a conserved ion channel termed Orco, which is obligatory for location and maintenance of ORs to dendritic cilia of antennal olfactory receptor neurons (ORNs). It is not resolved yet, whether odorant binding to ORs opens OR-Orco receptor ion channel complexes not only in heterologous expression but also in the intact insects Alternatively to ionotropic transduction, evidence is accumulating for metabotropic odor transduction, suggesting that insect ORs couple to G-proteins. Resulting second messengers gate various ion channels underlying the sensillum potential response eliciting a characteristic sequence of AP responses. Since it remained unclear to what extend and at what time Orco opens after odor-OR-binding, we examined the effects of the specific Orco antagonist OLC15 and the amilorides MIA and HMA on bombykal transduction in the crepuscular hawkmoth Manduca sexta. While both amilorides decreased the pheromone-dependent sensillum potential amplitude and the phasic pheromone response OLC15 had no effect. Instead, OLC15 strongly decreased spontaneous activity, increased the latency of the pheromone response and decreased the late, long-lasting pheromone response seconds to minutes after pheromone application. We concluded that both amilorides and OLC15 affected different targets at different phases of a metabotropic pheromone transduction cascade during the course of the day. In contrast to both amilorides Orco is not involved in the primary events of pheromone transduction. Rather, Orco acts as a voltage- and apparently also second messenger-gated pacemaker channel in ORNs controlling the membrane potential and hence threshold and kinetics of the pheromone response.

S24 - High fidelity transmission of temporal stimulus cues in the insect olfactory system.

Paul Szyszka, Jacob Stierle, Alpha Renner, Christoph J Kleineidam, Brian Smith, and Giovanni C Galizia

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Insects primarily rely on olfaction to locate resources such as food or mating partners. Tracking down an odor source poses a particular challenge: small scale air dynamics cause odors to occur in turbulent plumes in which they intermingle among themselves and with background odors, creating temporally complex patterns of different concentrations and odor mixtures. How do insects detect, recognize and find the right odor source in such a complicated odor environment? Odorants from the same sources fluctuate synchronously, whereas odorants from spatially separated sources fluctuate asynchronously. I will present evidence that the insect olfactory system is capable to resolve fast odor plume dynamics, which would allow it to detect whether odorants originate from the same or separate sources. Extracellular recordings from Drosophila olfactory receptor neurons show that (1) they can respond to odorants within less than 3 milliseconds (they are fast), and (2) follow repetitive odorant pulses above 100 Hz (they have high temporal resolution). Calcium imaging in the honey bee antennal lobe shows that projection neurons can resolve few millisecond asynchrony in the arrival of two odorants. Correspondingly, behavioral experiments in honey bees show that few millisecond asynchrony between odorants facilitates the perceptual segregation of concurrent odorants. These data show that temporal jitter between stimuli may help telling odor objects apart. Thus, olfactory source segregation shares common principles with visual and auditory source segregation in humans: we also use temporal jitter between stimuli to tell objects apart.

S25 - Structure and function of olfactory circuits in Drosophila.

Silke Sachse

Max Planck Institute for Chemical Ecology, Jena, Germany

Species of quite diverging animal phyla with an advanced olfactory system share an important similarity, which is the presence of olfactory glomeruli. During the last decades the wiring properties of these spherical compartments has been elucidated in great detail while only little is known about the numerical neuronal composition of individual glomeruli. The lack of exact numbers leads to a common basic assumption of glomerular uniformity, although different glomeruli do not accomplish a uniform function. In order to scrutinize whether each glomerulus possesses a unique neuronal architecture or whether glomeruli are uniform structural units, we characterized the detailed neuronal architecture of individual glomeruli and correlated these anatomical features with their functional properties in the model organism Drosophila melanogaster. We report a complete quantitative mapping of all receptor-specific sensory neurons that innervate a certain glomerulus, including sexually dimorphic distributions and glomerular volumes. Our data disprove the so far assumed universal 30:1 convergence and demonstrates for the first time the impact of OSN number on glomerular dimensions. Moreover, we show sex-specific differences in neuron number and glomerular volume also for fruitless negative glomeruli. In addition, we demonstrate a glomerulus-specific projection neuron innervation. Finally, we correlate these morphological features with functional properties and provide evidence for a unique neuronal architecture of glomeruli encoding behavioral relevant odors.

S26 - Optical dissection of pre- and postsynaptic plasticity in the olfactory system of Drosophila melanogaster.

André Fiala and Ulrike Pech

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Drosophila represents a key model organism for dissecting neuronal circuits of the olfactory pathway that underlie innate and learned odor-evoked behavior. This task is limited by a lack of tools to monitor physiological parameters of spatially distributed, central synapses in identified neurons. We generated transgenic fly strains that express functional fluorescence reporters targeted to either pre- or postsynaptic compartments. Presynaptic Ca2+ dynamics were monitored using synaptophysin-coupled GCaMP3; synaptic transmission was monitored using red fluorescent synaptophysin-pHTomato; and postsynaptic Ca2+ dynamics were visualized using GCaMP3 fused with the postsynaptic matrix protein, dHomer (Pech et al. 2015, Cell Rep. 10:2083–95). Using two-photon in vivo imaging of olfactory projection neurons, odor-evoked activity across populations of synapses were visualized in the antennal lobe and the mushroom body calyx. We have used these synaptically targeted sensor proteins to monitor synaptic plasticity caused by extended periods of odor exposure that typically induces behavioral adaptation. Prolonged odor exposure caused odor-specific and differential experience-dependent changes in post- but not presynaptic activity of projection neurons in the antennal lobe. Possible mechanisms mediating this adaptation to prolonged odor exposure are discussed. Further strategies how synaptically targeted fluorescence sensors can be used to analyze mechanisms of neuronal plasticity in the context of olfactory processing and learning will be presented.

S27 - Odour coding and organisational logic of the lateral horn.

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MRC LMB, Division of Neurobiology, Cambridge, United Kingdom

Odour information from the antennal lobes is relayed to the mushroom body (MB) and lateral horn (LH). The MB supports learning and memory while the LH is proposed to mediate innate olfactory behaviour. Although this dichotomy is broadly supported by behavioural and anatomical work, some data indicate the MB is required innate olfactory behaviour. Conversely recent data suggest connections between the MB and LH may be important for expression of learned behaviour. Therefore to understand fully learned and innate behaviour we must understand the circuit logic of both higher olfactory centres. Recent published work from our group characterised fruitless –positive sexually dimorphic LH neurons that respond selectively to the pheromone cVA in one sex or the other (Kohl, Ostrovsky et al, Cell, 2013). One cell class was extremely narrowly tuned while others responded to both pheromone and general odours. Our studies of generalist LHNs began with a split Gal4 screen. LHNs are anatomically very diverse including both local neurons and output neurons terminating in many different protocerebral regions, including zones of co–-convergence with MB output neurons. We identified 26 coarse cell classes of output neuron and 3 classes of local neuron. Fine scale analysis subdivided this into 85 cell clusters with distinct cell body positions, axon tracts or termination sites. We made > 400 in vivo whole cell patch recordings of LHNs defined by our driver lines, testing at least 36 odours/cell and filling cells for detailed anatomy. Similar data for second order projection neurons (PNs) are not available, so we also recorded their response to the same odours for comparison. The main dataset consists of 36 test odours for 257 output LHNs, 82 local LHNs and 75 PNs. Output LHNs typically have low baseline firing rates (median 0.1 Hz) while local interneurons fire at 10 times that rate (see also Fisek and Wilson, Nature, 2014). LHN odour tuning patterns could be divided into stereotyped functional classes; these classes exactly matched anatomically defined subsets. We found evidence for organisation by odour functional group. Intriguingly there is wide variation in tuning from ultra-sparse (1/40 odours) to very broad (almost all odours). In comparison to PNs, output LHNs fired fewer spikes, were somewhat more broadly tuned but had a higher signal to noise. In conclusion, generalist LHNs, like their pheromone –responsive counterparts, have diverse but genetically stereotyped odour response properties, consistent with a role in innate behaviour.

S28 - Odorant dominance in olfactory mixture processing: what makes a strong odorant?

Martin Giurfa

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The question of how animals process stimulus mixtures remains controversial as opposing views propose that mixtures are processed analytically, as the sum of their elements, or holistically, as unique entities different from their elements. Overshadowing is a widespread phenomenon that can help deciding between these alternatives. In overshadowing, an individual trained with a binary mixture learns one element better at the expense of the other. Although element salience (learning success) has been suggested as a main explanation for overshadowing, the mechanisms underlying this phenomenon remain unclear. We studied olfactory overshadowing in honey bees to uncover the mechanisms underlying olfactory-mixture processing. We provided the most comprehensive dataset on overshadowing to date based on 90 experimental groups involving more than 2700 bees trained either with 6 odorants or with their resulting 15 binary mixtures. We found

that bees process olfactory mixtures analytically and that salience alone cannot predict overshadowing. After normalizing learning success, we found that an unexpected feature, the generalization profile of an odorant, was determinant for overshadowing. Odorants that induced less generalization enhanced their distinctiveness and became dominant in the mixture. We thus uncovered features that determine odorant dominance within olfactory mixtures and refer this phenomenon to differences in neural activity both at the receptor and the central level in the insect nervous system.

PARALLEL SYMPOSIUM I: UMAMI: BEYOND THE TASTE FOR A HEALTHIER LIFE.

PS1 - Overview of umami flavor and health.

Ole G Mouritsen

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Diet and lifestyle have an impact on the burden of ill health and non-communicable ailments such as cardiovascular disease (including hypertension), obesity, diabetes, cancer, and certain mental illnesses. The consequences of malnutrition and critical unbalances in the diet with regard to sugar, salt, and fat are becoming increasingly manifest in the Western world and are also gradually influencing the general health condition for populations in developing countries. In this overview talk I will highlight the lack of deliciousness and umami flavor in prepared meals as a possible reason for poor nutritional management and excess intake of salt, fat, and sugar. I argue that a better informed use of the current scientific understanding of umami and its dependence of the synergetic relationship between monosodium glutamate and certain 5′-ribonucleotides and their action on the umami taste receptors will not only provide better-tasting and more flavorsome meals but may also help to regulate food intake, in relation to both overeating and nutritional management of elderly and sick individuals.

PS2 - Clinical assessment of taste disorders.

Thomas Hummel

Smell and Taste Clinic, Department of ORL, TU Dresden, Dresden, Germany

Gustatory disorders are rarely reported spontaneously. Thus, when interviewing a patient it seems mandatory to specifically ask questions related to this sense, separating gustatory from olfactory dysfunction. Clinical assessment should include the complete examination of the cranial nerves and, in particular, gustatory testing using whole mouth techniques and regional testing. Further functional tests are available through electrophysiological recordings or functional MRI. Structural imaging (MRI and CT) enables the delineation of neuroanatomical structures involved in the processing of gustatory sensations. Gustatory disorders appear as quantitative (absence of taste – ageusia, diminished sensitivity - hypogeusia) and more often as qualitative dysfunction (distortion of gustatory sensations - dysgeusia). These symptoms can result from damage at any location of the neural gustatory pathway from the taste buds via the peripheral (CN VII, IX, X) and central nervous system (brain stem, thalamus) to its representation at the cerebral cortex. Pathogenetically, a large number of causes has to be considered, e.g., drugs, cerebrovascular disorders, tumors, head trauma, neuropathy, epilepsy, or major depression. Due to the broad differential diagnostic considerations it is essential to look for additional neurological signs and symptoms. Treatment must relate to the underlying cause, e.g. in drug-induced taste abnormalities. Zinc may be useful in idiopathic dysgeusia.

PS3 - Umami taste and health status.

Aytug Altundag

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There have been many researches about umami and other four basic tastes from the time umami taste identified and still emerges as an issue that being discussed. The changes in four basic tastes due to many circumstances and painful diseases have been investigated in many studies, whereas there has been limited studies on umami because of its various perceptions on tongue and palatal area, also because of cultural differences in the taste of umami. Today, because of the intense contribution to the perception of the taste of umami flavor, that brings a high level of impact on quality of life as well. Which leads to problems in the perception of taste in the mouth exceptions made changes in the perception of umami taste changes in the salivary secretions are provided with umami taste stimulations. Laryngopharyngeal reflux disease has effects such as dental erosion and halitosis but also has effects on the taste of umami taste perception that occur deterioration in these patients also appears to be a decreased quality of life parameters. However, the change in the anatomy of the oral cavity causing snoring surgery method performed anterior palatoplasy also arises changes in the sensitivity of the umami taste. In particular, the stimulation must necessarily be informed in detail and will consist of patients with postoperative changes in the perception of taste umami taste and a reduction in sensitivity may occur after resections in the palate.

PS4 - The important role of umami taste in oral and overall health and a possible genetic diagnosis for umami taste disorders.

Noriaki Shoji, Shizuko Satoh-Kuriwada, Takashi Sasano

Tohoku University, Graduate School of Dentistry, Department of Oral Diagnosis, Sendai, Japan

In our taste clinic, we sometimes experience elderly patients who complain of persistent subjective impairment of umami taste with preservation of the other four basic taste sensations (sweet, salty, sour, and bitter). Such patients suffer from appetite and weight loss, resulting in poor overall health. After appropriate treatment, such patients with umami taste disorder showed remarkable improvements in their appetite and weight with normalization of their umami taste sensation. These results indicate that the umami taste sensation is very important to the maintenance of good health in the elderly. Thus, the diagnosis of umami taste disorders should be important, however, a reliable umami taste sensitivity test that can precisely measure taste sensitivity at different sites in the oral cavity has yet to be established. Consequently, there is no detailed information regarding impaired umami taste sensitivity in patients with taste disorders. We recently developed a method using monosodium glutamate (MSG) as a test solution to assess umami taste sensitivity because Japanese commercial product for taste test assesses only four of the five basic tastes: sweet, salty, sour, and bitter. The filter paper discs (5 mm dia.) were soaked in aqueous MSG solutions (1, 5, 10, 50, 100 and 200 mM), then placed on three oral sites innervated by different taste nerves. The lowest concentration participants correctly identified was defined as the recognition threshold (RT) for MSG. Our sensitivity test showed good reproducibility for inter- and intra-observer variability, and conclusively high diagnostic performance for discriminating between normal taste function and umami taste disorders according to established cut off values (PLoS One, 2014). However, this test is not necessarily useful for the patients who cannot express their taste sensation induced by the tastant, such as patients with dementia because of the subjective nature of the test. Consequently, an objective assessment method for umami taste loss should be established. To develop an objective umami taste test, we evaluated the expression of the umami receptor genes in the tongue. Tissue samples were collected from healthy volunteers by scraping the foliate papillae of the tongue. Immunocytochemistry for gustducin, a taste-cell-specific G protein, showed the samples contained taste cells. Quantitative real-time PCR using specifically designed primers enabled amplification and quantification of expression of the umami taste-related genes [gustducin (GNAT3), T1R1, T1R3, and mGluR1] without mispriming and primer-dimer artefacts. Additionally, this method could detect alterations in the gene expression of the umami taste-related genes, particularly T1R1 and T1R3, in response to MSG. This report is a preliminary study for clinical use, but the genetic taste assessment would be available for the objective diagnosis of umami taste disorders (Oral Diseases in press, 2015).

PARALLEL SYMPOSIUM II: FROM RECEPTORS TO PERCEPTION.

PS5 - Large scale transcriptional profiling of chemosensory neurons identifies receptor-ligand pairs in vivo.

Ivan Rodriguez

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In mammals, olfactory perception is based on the combinatorial activation of G protein–coupled receptors. Identifying the full repertoire of receptors activated by a given odorant in vivo, a quest that has been hampered for over 20 years by technical difficulties, would represent an important step in deciphering the rules governing chemoperception. We found that odorants induced a fast and reversible concentration-dependent decrease in the transcription of genes corresponding to activated receptors in intact mice. On the basis of this finding, we developed a large-scale transcriptomic approach to uncover receptor-ligand pairs in vivo. We identified the mouse and rat odorant receptor signatures corresponding to specific odorants. Finally, we found that this approach, which can be used for species for which no genomic sequence is available, is also applicable to non-vertebrate species such as Drosophila.

PS6 - Specific olfactory receptors mediate musk odor perception.

Kazushige Touhara and Mika Shirasu

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Musk odorants are widely utilized in cosmetic industries because of their fascinating animalic scent. Muscone, one of the major musk compounds, possesses a unique macrocyclic ketone structure. Muscone is originally discovered from the stink glands of male musk deer, and has pheromonal effects on musk deer. It also affects human's physiological state. Natural muscone is expensive, and capturing musk deer is currently prohibited, thus hundreds of synthetic musk odorants have been developed until now. Although the quality of the odor of various musk compounds is similar, the structures differ considerably. From these viewpoints, the mechanism by which musk aroma is perceived in the olfactory system is of great interest. Recently, we reported that muscone activated a few glomeruli in the mouse olfactory bulb, suggesting that muscone is recognized by one or two olfactory receptors (ORs) in mice. By using a retrograde neural labeling technique, we identified a muscone-responsive mouse OR, MOR215-1 and also found a human muscone receptor, OR5AN1 that shows the highest amino acid identity to MOR215-1 (Shirasu 2014). Here we found that an orthologue of human OR5AN1 has expanded in the mouse lineages, generating six highly similar OR genes in mice. Out of these, two musk ORs, MOR215-1 and

MOR214-3, responded to muscone in the luciferase assay systems. The threshold concentration of muscone for MOR214-3 was approximately 100-fold higher than that for MOR215-1. According to behavioral assays (odor-finding test), MOR215-1 knockout mice couldn't detect muscone at the low concentrations. Therefore, MOR215-1 is likely the major musk receptor in mice. To elucidate the characteristics of musk ORs in more detail, we evaluated the structure-activity relationships of musk ORs in mouse and five primate species with various musk odorants and structurally-related compounds by the luciferase assay systems in HEK293 cells. These ORs exhibited unique ligand spectra to musk compounds. Interestingly, some of them including the human OR5AN1 responded to nitro musks that have distinct chemical properties from muscone. The structure-activity relationships of OR5AN1 are in good agreement with our sensory perception, that is to say, odorants that activate OR5AN1 tend to have musk odor. Moreover, the comprehensive screening of almost all human ORs to muscone indicated that only two ORs including OR5AN1 were activated by muscone. Additionally, our preliminary data suggest that OR5AN1 genotypes partly explain the sensitivity difference to muscone. All these results suggest that OR5AN1 is crucial to the perception of musk odor in humans. Our current study clearly shows a case that a single OR contribute to odor perception in mice and human.

PS7 - Contribution of single olfactory receptors to odor perception.

Thomas Bozza, Adam Dewan, Annika Cichy, and Jingji Zhang

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Volatile ligands are detected in the mammalian main olfactory system by a large repertoire of G protein-coupled receptors. In mice, these include over 1,000 canonical odorant receptors (ORs) and a much smaller family of 14 Trace Amine-Associated Receptors (TAARs). We are using gene targeting, electrophysiology, optical imaging, and behavior to characterize the ligand response properties of ORs and TAARs in vivo, and to quantify how individual receptors contribute to odor perception. I will present evidence that the TAARs mediate high sensitivity detection of volatile amines and that individual TAAR genes contribute significantly to odor detection and valence. Our work permits us to measure how ligand recognition at the receptor level impacts odor perception at the behavioral level.

PS8 - Distinct molecular receptors mediate sweet and fatty acid taste through overlapping sets of taste neurons.

Hubert Amrein, Yan Chen, and Ji-Eun Ahn

Texas A&M University Health Science Center, Department of Molecular and Cellular Medicine, College Station, USA

Carbohydrates, proteins and fats constitute the three main classes of nutrient resources in the diet of most animals. While significant progress in our understanding of how sugars and amino acids are sensed by taste systems, little is known about the molecular and cellular basis of fatty acid (FA) sensing in either mammals or insects. Behavioral genetic studies have shown that Drosophila exhibit an appetitive behavioral response by extending their proboscis when their tarsal taste sensilla are stimulated with FAs. This proboscis extension reflex (PER) has been well established as the initial motor action necessary for feeding in many insects, and it has been primarily employed as the standard behavioral feeding assay for Drosophila to sugars. Here, we use Ca2+ imaging experiments and behavioral analyses to show that sweet sensing gustatory receptor neurons are activated both by sugars and FAs, and that sugar-blind flies lacking all eight sugar receptor (sugar Gr) genes show normal PER and cellular responses to FAs. We also show that two Ionotropic receptor (Ir) genes, Ir25a and Ir76b, are abundantly expressed in numerous gustatory receptor neurons of the fly, including most, but not all sweet gustatory receptor neurons. Mutations in either gene cause a loss of appetitive behavior towards FAs, reflected by a severe reduction in PER frequency. Moreover, Ca2+ imaging experiments show that activation of sweet gustatory receptor neurons is dependent on both the Ir25a and Ir76b genes. Coincidently, we discovered that a distinct set of Ir25a/76b expressing neurons responds to carboxylic acids (CAs), and that both genes are necessary for the cellular response of these neurons to these chemicals. CAs have no major nutritional value for Drosophila, but they are important auxiliary components preferred by females for the deposition of eggs. Thus, our studies provide direct evidence that perceptions of FAs and CAs are mediated through distinct sets of gustatory receptor neurons. In consideration of previous studies of Ir genes in the olfactory system, we suggest that functional FA and CA receptors are multimeric complexes that share common Ir core components (Ir25a and Ir76b), but are characterized by distinct Ir subunits that define the different chemical categories that these neuronal subtypes recognize.

PS9 - Predicting human odor perception from olfactory receptor activation.

Casey Trimmer, Jason R Willer, Andreas Keller, Leslie B Vosshall, Nicholas Katsanis, Hiroaki Matsunami, and Joel D Mainland

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Although we know that the olfactory system uses a combinatorial code to represent odors, it is unclear how loss-of-function in a single olfactory receptor (OR) will alter perception. To date, we know of only five cases that specifically link genetic variation in an OR with alterations in perception, making it difficult to generalize to the entire 400-member OR family. To

answer this question, we asked 321 human subjects to rate the intensity and valence of 68 odors at two concentrations and then sequenced each subject's OR subgenome. Variation in a single OR was significantly associated with changes in perception for 20 of the 68 tested odors (29%) ($p < 0.05$, with FDR correction), suggesting that the combinatorial code is not highly redundant. We identified significant associations with 43 polymorphisms in 27 different genes. We then set out to identify the causal OR underlying each association using an in vitro assay. We were able to identify responsive ORs for roughly 50% of our significant associations. For many of these associations, human subjects with genetic variants that reduce odor response in vitro rated the intensity of the odor to be lower than subjects with more functional alleles. More rarely, in vitro function also correlated with perceived valence. In addition, despite the fact that our study was designed to identify cases in which a single olfactory receptor is relevant to perception, we can use this data to examine the contribution of multiple ORs to odor perception. For example, although OR10G4 genotype is a good predictor of guaiacol perception, considering the genotypes of all ORs responsive to guaiacol in cell culture improves our prediction of guaiacol perception. These results provide a potential approach for identifying behaviorally relevant OR/odor interactions via a heterologous assay and demonstrate that, despite the combinatorial nature of the olfactory code, alterations in a single odorant receptor can have a significant effect on odor perception.

PARALLEL SYMPOSIUM III: THE BIOLOGICAL BASES OF OLFACTORY PERCEPTION AND LEARNING.

PS10 - Lateralization of odor memory in rats.

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Asymmetry, or cerebral lateralization, allows functional specialization of bilateral brain regions and has been described in humans for such diverse functions as perception, memory and emotion. The human olfactory system also demonstrates task-dependent functional asymmetry. For example, odor recognition and familiarity are heavily mediated by right hemisphere structures such as the orbitofrontal cortex (OFC) and piriform cortex (PCX), while ratings of odor hedonics are more dominated by the left OFC. However, the mechanisms of olfactory system asymmetry and lateralization of function are unknown. In this presentation, we will describe recent work exploring asymmetry in the rodent PCX and OFC over the course of training in a two-alternative, forced-choice odor discrimination task and reversal training. Local field potentials were recorded bilaterally in the anterior PCX and OFC in rats. Functional asymmetry emerged over the course of training in both regions, though the direction of asymmetry differed between regions. Initial learning evoked a transient bias (stronger evoked activity) in the left PCX, while both initial learning and reversal learning evoked a bias toward the right OFC. Furthermore, during early stages of acquisition, functional connectivity (coherence) between the left and right PCX decreased during task performance and returned as the animals mastered the task. This suggesting a transient hemispheric disconnection during initial learning. Together the findings suggest robust asymmetry in rat olfactory processing and will allow exploration into underlying neural mechanisms.

PS11 - Neurobiology of odor learning ontogeny within rat pups' ecological niche.

Regina Sullivan

Emotional Brain Institute, Child & Adolescent Psychiatry, NKI, NYU School of Medicine, New York, USA

To support attachment to the caregiver, altricial infants such as humans and rats, must identify, learn, and remember their caregiver. As suggested by Bowlby in the 1950's, this rapid attachment learning must rely on a biological attachment system in the brain. While the infant attachment circuit has not yet been identified in the human, research in the rat is beginning to document the infant attachment neurobehavioral process, which is exquisitely suited to promote the infant-caregiver relationship. Foremost, pups have the enhanced ability to acquire learned preferences, and this behavior is supported by the hyperfunctioning locus coeruleus and experience-induced changes in the olfactory bulb and anterior piriform cortex. However, of equal importance, infants also have a decreased ability to acquire learned aversions/fear, and this behavior is facilitated through attenuated amygdala activity. Thus, odors paired with pain or pleasure result in a subsequent approach to that odor, but it also takes on characteristics of the maternal odor. Presumably, this attachment circuitry constrains the infant to form only preferences for the caretaker regardless of the quality of the care received. Once attachment is learned, the caregiver's sensory cues regulate the infant brain to alter behavior and facilitate infant interaction with the caregiver. First, we present data illustrating how the mother's social buffering of her pups' stress response during odor-pain learning blocks amygdala-dependent fear learning, inhibition of amygdala dopamine release and related gene expression to generate attachment learning. Second, we show how social referencing by pups of the mother's fear response can override social buffering to permit pups to learn fear in her presence. The pups learn a specific amygdala-dependent fear odor controlled by the fearful mother's ability to increase pups' corticosterone. Third, we show how odor cues associated with infant attachment continue to influence brain function into adulthood to alter social behavior and emotional processing through amygdala serotonin increase and glucocorticoid decrease.

PS12 - Cellular and molecular mechanisms underlying complex olfactory learning.

Edi Barkai

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Learning-induced enhancement in neuronal excitability is evident in hippocampal and piriform cortex pyramidal neurons following a complex olfactory-discrimination operant conditioning task. Such enhanced excitability is manifested in reduced spike frequency adaptation that results from reduction in the slow afterhyperpolarization (AHP), which develops after a burst of action potentials. AHP reduction is apparent throughout the pyramidal cells neuronal population. The post-burst AHP reduction is mediated by decreased conductance for a specific calcium-dependent potassium current, the sIAHP. This long-lasting reduction is dependent on persistent activation of the PKC and ERK second messenger systems. Similar long-lasting AHP reduction can be induced in-vitro by repetitive synaptic stimulation or by kainate application. Such activity-dependent AHP reduction is occluded by prior learning. Olfactory-learning induced enhanced neuronal excitability in CA1 pyramidal neurons is also accompanied by enhanced learning capability in a novel hippocampus-dependent task, the Morris water maze. We suggested that AHP reduction is the cellular mechanism that enables neuronal ensembles to enter into a state which may be best termed "learning mode". This state lasts for up to several days and its behavioral manifestation is enhanced learning capability in tasks that depend on these particular neuronal ensembles.

PS13 - A translational approach of olfactory episodic memory characteristics and circuits.

Nadine Ravel

University of Lyon, Lyon, France

In search for the mechanisms underlying complex forms of human memory such as episodic recollection, a primary challenge is to dispose of adequate animal models amenable to neurobiological investigation. Our group has recently developed a novel framework and paradigm that provides means to quantitatively evaluate the ability of rats to form and recollect a combined knowledge of What happened, Where and When/in Which context (referred to as episodic-like memory) after limited encounter of specific episodes in a situation as close as possible to a paradigm we recently developed to study episodic memory in humans. In the first part of my talk, I will present the two protocols in parallel insisting both on their proximity and divergence. In a second part, I will more focus on the results obtained in rodents. In our paradigm, rats have to remember odor-drink associations (What happened) encountered in distinct locations (Where it happened) within two different multisensory enriched environments (In Which context/occasion it happened) each characterized by a particular combination of odors and places. By analyzing licking behavior on each port, we characterized quantitatively individual recollection profiles and showed that rats are able even after limited exposure to the episodes, to incidentally form and recollect an accurate, long-term integrated episodic-like memory that can last up to 24 days. Placing rats in a contextually challenging recollection situation at recall revealed the ability for flexible use of episodic memory as described in humans. We further report that reversible inactivation of the dorsal hippocampus disrupts the animal's capacity to recollect the complete episodic memory. Finally, using cellular imaging of c-Fos and Zif268 brain activation, we reported that episodic memory recruits a specific, distributed network of hippocampal-prefrontal cortex structures that correlates with the accuracy of the integrated recollection performance. At the end of my talk, I will discuss some of the questions we would like to address in parallel taking advantage of what is possible to do in each model.

PS14 - Oxytocin facilitates synaptic plasticity of chemosensory inputs to the mouse medial amygdala.

Peter Brennan and Nick Cole

University of Bristol, School of Physiology and Pharmacology, Bristol, United Kingdom

The medial amygdala is a central site for integration of information about social identity in a variety of species including mice. Its major input comes from the accessory olfactory bulb, conveying information sensed by the vomeronasal organ. However, the medial amygdala also receives a significant and convergent input from the ventral region of the main olfactory bulb. This convergence of inputs provides a potential anatomical basis for the association of main olfactory and vomeronasal information, such as that underlying darcin-mediated learning of the urinary volatiles signature of individual mice. This study investigated whether long-term potentiation (LTP) could be induced at the input synapses to the medial amygdala and whether it could be enhanced by oxytocin, which has been implicated in the formation of social memory in mice, via an action at the medial amygdala. In vitro electrophysiological recordings were performed using parasagittal slices through the medial amygdala of BALB/c female mice. Stimulation of the axons of the olfactory tract, anterior to the medial amygdala, resulted in a postsynaptic potential that could be recorded in the medial amygdala. The postsynaptic origin of this potential was confirmed by its paired-pulse facilitation and its dependence on extracellular Ca2+. The postsynaptic potential was also abolished in the presence of 30μM CNQX demonstrating that it was likely to be mainly mediated by AMPA glutamatergic transmission. High frequency stimulation (100 Hz for 1 second) of the input axons resulted in an enhancement

of the postsynaptic response by around 25% of baseline (n=6), which was maintained for over an hour, indicating LTP of the input synapses. Reduction of the high frequency stimulation to 40 pulses at 100Hz resulted in only short-term potentiation of the postsynaptic response, which returned to baseline within 30 minutes (LTP in 0/5 slices). However, delivery of this sub-threshold 40-pulse stimulus in the presence of 2μM oxytocin resulted in LTP in 5/5 slices (mean of 22.3 ± 3.6% after 1 hour). Oxytocin did not affect the basal level of synaptic transmission before potentiation, suggesting a similarity with oxytocin-enhanced potentiation in the CA1 region of the hippocampus. These findings of oxytocin-enhanced synaptic plasticity are consistent with the hypotheses that these input synapses might be a synaptic locus for association of chemosensory input in the medial amygdala that underlies oxytocin-dependent social recognition.

PS15 - Neonatal exposures to cat odors in the house mouse produce sensitization and habituation to target signals at the behavioral level but not at the hormonal.

Vera Voznessenskaya, Ilya Kvasha, Tatiana Laktionova, Artyom Klinov, and Maria Klyuchnikova

A.N.Severtsov Institute of Ecology and Evolution, Moscow, Russia

Chemosensory detection is a very important aspect for predator avoidance strategy for many mammals including the house mouse. Odors from carnivores may elicit fear-induced stereotypic behaviors, change activity patterns and feeding rate, and affect the neuroendocrine system, reproductive behavior, and reproductive output in potential prey. Domestic cat is the most specialized predator to the house mouse. Previously we examined the influence of the species specific compound from the cat urine L-felinine on the behavior, neuroendocrine responses and on the reproductive success in mice. Exposures of mice Mus musculus to L-felinine (0.05%) significantly affected reproductive success as well as caused clear corticosterone response (Voznessenskaya, 2014). Neonatal exposure to odorants may influence the function of the olfactory system of an animal and produce changes in its responses to these odorants later in life. Our earlier studies showed that regardless of the odorant (conspecific or heterospecific urine, androstenone), early exposure to it resulted in an increase in a rodent's sensitivity to that stimulus (Voznessenskaya et al. 1995). Exposure to the odorant during two weeks after the pups opened their eyes appeared to induce the greatest level of sensitization, suggesting a sensitive period to such stimuli (Voznessenskaya et al. 1999). Thus, the specific aim of our current study was to examine whether early olfactory experience of mice with chemosignals of cats during "critical" period for sensitization to odors may modulate behavioral or neuroendocrine responses to the target cues later in adulthood. We used three basic approaches: behavioral, hormonal and immunohistochemical. Olfactory thresholds to cat urine and L-felinine were measured with an automated olfactometer (Knosys, USA). Fecal specific glucocorticoid metabolites and plasma corticosterone were monitored using an ELISA technique. Behavioral patterns were analyzed using an open field paradigm (two different modifications). Exposures of mice to cat chemical cues (urine or L-felinine) during "critical period" significantly lowered the olfactory thresholds (n=10, $p<0.05$; n=10, $p< 0.01$) which is adaptive for detection of the predator under natural conditions. Immunohistochemical studies showed elevated fos-immunoreactivity in accessory olfactory bulb in response to stimulation with L-felinine (n=8, $p< 0.001$) in mice neonatally exposed to the target odor relative to the controls. Neonatal exposures also decreased (n=22, $p< 0.01$) patterns of passive-avoidance behavior to cat odors and elevated significantly investigatory activity (n=22, $p< 0.01$). At the same time corticosterone response to cat urine/L-Felinine stayed unchanged (n=10, $p< 0.01$) indicating the innate nature of the response. Early olfactory experience with cat odors produced dissociation in responses to these odors later in the life at the behavioral level and at the hormonal level.

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PARALLEL SYMPOSIUM IV: TASTE RECEPTORS MONITORING THE BODY'S INTERNAL AND EXTERNAL ENVIRONMENT.

PS16 - Specificity of Connectivity in Taste Buds: A draft connectome.

Thomas Finger[1,2], Robert S Lasher[1,2], Ruibiao Yang[1,2], Grahame J Kidd[3], and John C Kinnamon[1,4]

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Every taste bud contains a variety of taste cells each of which expresses transduction machinery tuned to one of the primary taste qualities: sweet, umami, bitter, sour or salty. How these differently tuned taste cells are connected to the different taste nerve fibers innervating each bud is unclear. In an effort to address this question, we have utilized serial blockface scanning electron microscopy (sbfSEM) to produce high resolution image stacks with ultrastructural resolution encompassing 2–4 taste buds of the circumvallate papilla from mice. By manual identification and segmentation of key elements within this image stack, we have followed individual nerve fibers and identified the specialized points of contact between the nerve fibers and the different cells within the taste buds. Taste cells transducing umami, sweet or bitter are all Type II taste cells and therefore are

not distinguishable from each other in our initial preparations. In contrast, sour-transducing cells are readily identifiable Type III cells enabling us to ask the question whether any nerve fiber forms specialized contacts with both Type II (umami, sweet, bitter) and Type III (sour) taste cells. If so, then taste is not encoded in a strict labeled-line system where each axon conveys information about only a single taste quality. Specialized contacts between Type III cells and nerve fibers are canonical synapses with readily identified presynaptic vesicles and membrane specialization on the pre- and post-synaptic faces of the contact. Type II cells form no such contacts, but do display an alternative specialization consisting of large atypical mitochondria, and thickened parallel membranes of both the taste cell and adjacent nerve fiber. Our 3D reconstructions also show that the nerve fibers not only contact the Type II taste cells, but form flattened enlargements across the face of the taste cell at the point of specialized contact. In our initial samples, we have traced a total of 17 nerve fibers which contact a total of 6 Type II cells and 1 Type III cells. Although a given nerve fiber may form specialized, presumed synaptic, contacts with multiple taste cells, in no case has an individual nerve fiber formed specialized contacts with both Type III and Type II cells. This indicates that information about sour taste is conveyed over different fibers than information about other modalities. Whether the same principle holds true for the Type II qualities of umami, sweet, and bitter, awaits further study with transgenically labeled cell populations.

PS17 - Developing taste bud cells are motile.

Marika Kapsimali[1], M Soulika[1], AL Kaushik[1], B Mathieu[1], R Lourenço R[1] AZ Komisarczuk[2], S Romano[1], A Jouary[1], A Lardennois[1], N Tissot[3], S Okada[4], K Abe[4], and TS Becker[2]

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The assembly of differentiating cells into a taste sensory organ is a poorly-defined process. In jawed vertebrates, the taste sensory organs (taste buds) are composed of distinct cell types that detect and transduce specific taste qualities including salty, bitter, sweet, umami and sour. Taste-bud cells differentiate from ectoderm or endoderm-derived epithelial progenitors localized in the proximity of the forming organs. To get insight into the process through which differentiating cells form the taste-bud organ, we made timelapse in live zebrafish larvae. We found that early (fgf8a expressing) and late (plcß2 expressing, Type II) differentiating cells are motile from the epithelium to the site of the forming organ but also between neighbouring developing taste buds. In addition, ascl1a activity in the 5-HT /Type III cell is required to direct early differentiating cells towards the developing taste bud, to maintain them within the organ, and control the appropriate number of organs. We conclude that the formation of taste-bud organs is a highly dynamic process relying on the diverse modes of motility of the differentiating cells.

PS18 - Cholinergic brush cells: Detectors of mucosal infections?

Kummer Wolfgang, Gabriela Krasteva-Christ, Aichurek Soultanova, and Klaus Deckmann

Institute of Anatomy and Cell Biology, Justus-Liebig-University Giessen, Giessen, Germany

Early electron microscopical studies have identified a rare, specialized epithelial cell in lower airway and gastrointestinal mucosal surfaces which is characterized by an apical tuft of stiff microvilli containing the structural protein villin. Due to this morphological feature, these cells have been termed "brush cells". Cells with similar, albeit not entirely identical ultrastructure have been described in the nose. Their function remained enigmatic over decades, until functional expression of the canonical bitter and umami taste transduction cascade was noted in these cells. These cells utilize taste receptors to detect bacterial products such as quorum sensing molecules from Pseudomonas aeruginosa. Upon stimulation, these cells release acetylcholine and evoke both paracrine and, via excitation of sensory nerve terminals, general reflexes. We, hence, hypothesized that these cells serve as sentinels monitoring the mucosal lining fluid for ingression of potentially harmful content and initiating protective reflexes. Based on this concept, the presence of similar cells was predicted and validated at anatomical sites where they had not been reported before by conventional morphological techniques, including the auditory tube (entrance to the middle ear), the ocular conjunctiva (entrance to the lacrimal canalicular system), and the urethra (entrance to the urogenital tract). Most recently, such a cellular entity was also detected among thymic medullary epithelial cells. Currently, functional characterization of these cell populations is most advanced in the airways and urethra. In the latter, these cells serve as polymodal (bitter and umami) chemosensory cells, respond to uropathogenic bacteria, and initiate a "cleaning" micturition reflex upon stimulation. Their dysfunction might be related to a higher risk of urinary tract infection, and their endogenous cholinergic feedback loops appear to be a promising target for conditions of urge and frequency (overactive bladder).

PS19 - Bitter taste receptors in the brain.

Meyerhof Wolfgang[1], Anja Voigt[1], Antje Stolzenburg[1], Kristina Lossow[1], Sandra Hübner[1], Sabine Frenzel[1], Jonas Töle[1], and Ulrich Böhm[2]

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Vertebrates recognize bitter substances by means of oral G protein-coupled taste receptors of the taste 2 receptor family (Tas2r). Yet Tas2rs often have been detected outside the taste system in the central nervous, respiratory, cardiovascular, gastrointestinal, male reproductive, excretory, and immune system as well as in blood cells and mesenchymal tissues. Although their function in the non-gustatory tissues remains relatively poorly understood extraoral Tas2rs are considered as novel alternative drug targets. We report here an initial analysis of Tas2rs in the mouse brain. Systematic quantitative PCR for all 35 mouse Tas2rs demonstrated that a subset of 25 receptors is expressed in the mouse brain. This contrasts the situation in the mouth where all 35 Tas2rs are present. The expression patterns for the 25 receptors in various brain areas were very similar and related to the chromosomal location of the Tas2r genes. Further studies propose that in the brain only one Tas2r gene is expressed per cell which differs fundamentally from taste cells which express several Tas2rs. Thus, the data indicate that Tas2r gene regulation differs between oral and brain tissues. Expression levels of Tas2rs in brain tissues were generally low preventing visualization of Tas2r expressing cells in most cases. However, we successfully detected a few Tas2rs with high mRNA levels by in situ hybridization suggesting that Tas2rs are expressed by neurons. Tas2r expression in neurons was confirmed in genetically engineered mice in which activation of the Tas2r131 locus leads to expression of GFP. Distribution of GFP is widespread occurring in numerous areas. GFP positive cells are particularly abundant in the hypothalamus, thalamus, periaqueductal grey, and colliculi. Such cells were also present in comparatively high numbers in the medulla oblongata and less frequent in the hippocampus, olfactory bulbs and cerebral cortex. GFP exclusively colocalizes with neuronal marker but not with markers for astrocytes, oligodendrocytes, and microglial cells. Using markers for neurochemical coding we found Tas2r131 expression in cholinergic, dopaminergic, noradrenergic, and glycinergic projection neurons and in calbindin interneurons but not in serotonergic and GABAergic neurons. For the time being Tas2r functions in the brain remain unknown. However, the expression of Tas2rs in different types of neurons and their presence in numerous brain areas strongly suggest that bitter taste receptors fulfil multiple functions in the central nervous system.

PS20 - TAS2Rs - A new class of functional G protein-coupled receptors in heart.

Walter Thomas

The University of Queensland, School of Biomedical Sciences, Brisbane, Australia

G protein-coupled receptors (GPCRs) are mediators of cardiac function and targeted for the treatment of cardiovascular disease. We have provided the first evidence that GPCRs normally involved in taste (specifically the Tas2Rs) are expressed in human and rodent heart. Using a combination of molecular, physiological and pharmacological approaches, several members of this GPCR family have been identified in heart, cloned and their activating ligands identified. Further experiments in human and rodent hearts have demonstrated profound effects on cardiac contractility. The novel cardiovascular effects of these ligands are of significant interest, and open opportunities to investigate unappreciated aspects of heart biology.

PS21 - An extraoral taste circuit integrates nutritional state with sensory information for persistent sugar ingestion in Drosophila.

Nilay Yapici, Christian Schusterreiter, Leslie B Vosshall

The Rockefeller University, New York, USA

To survive and adapt to environmental changes, animals need to optimize the amount and quality of food consumed. Food intake is regulated in the nervous system by evaluating chemosensory information and nutritional state of the animal. Taste cells in oral and extraoral sensory organs transmit sensory information to higher order neural circuits that mediate food intake decisions. Here we investigated the function of an extraoral taste circuit in the fly Drosophila melanogaster, and identify sugar sensitive pharyngeal taste neurons that communicate food quality to a compact neural circuit, which integrates nutritional status and taste information to drive persistent food intake. We developed an automated real-time food intake assay, Expresso, which measures individual meal-bouts with nanoliter resolution, and use this system to show that flies regulate their food intake based on their nutritional state and the quality of the food presented. Using intersectional genetics, neural silencing and optogenetic activation, we identify ~10 local interneurons in the primary taste centre of the fly brain that are necessary and sufficient for food intake. Double-labelling experiments showed that extraoral taste neuron terminals overlap with IN1 interneuron arbours in the anterior subesophageal zone. We used the GFP reconstitution across synaptic partners (GRASP) method to show anatomical connectivity between IN1 neurons and the pharyngeal sensory neurons. Our work provides functional evidence for the existence of an extraoral taste circuit that integrates the nutritional status of the fly with taste sensory information to drive persistent sugar ingestion. These findings raise the possibility that extraoral food detection may provide a fast feedback mechanism to assess food quality and optimize food intake based on the internal state of an animal.

PARALLEL SYMPOSIUM V: CHEMOSENSORY INTERACTIONS AT DIFFERENT LEVELS: FROM MOUTH TO BRAIN

PS22 - Taste Mixture Interactions from a Sensory Perspective.

Harry Lawless and Johanneke Busch

Cornell University, Department of Food Science, Ithaca, USA

This talk will review the patterns of interactions of taste mixture components, as perceived by humans. Binary mixtures of the four classical tastes usually show mixture suppression. Suppression is defined as a decrease in the perceived intensity of one component when compared to the same concentration of that tastant when presented alone (unmixed). After reduction of one tastant's intensity, by adaptation for example, the other component of a mixture is "released" from suppression, i.e. returns toward the full intensity as perceived in the unmixed state. Other methods of blocking the taste of one component of a mixture show similar release effects. "Split-tongue" presentation of taste components also shows suppressive interactions, but not in all cases. The implications of these effects for the central vs. peripheral locations of inhibitory suppression mechanisms will be presented. If time permits, cases of synergistic interactions will also be discussed.

PS23 - Questions on peripheral interactions of taste and trigeminal sensitivity.

Annick Faurion

CNRS INSB NEUROPSI – NBS, Gif sur Yvette, France

Gustatory, somato-sensory oral information and olfaction, contribute to what the layerman names "Taste". These three exteroceptive chemoreception senses interfere at various levels in the brain. Here we focus on a description of the interactions between taste and somato-sensory signals. The interference between the chorda tympani (CT) and lingual trigeminal nerve (Vth) can be experimentally documented in the rodent as it is possible to record both nerves simultaneously while stimulating the tongue (Lugaz, 2004). Within the anterior part of the tongue, trigeminal afferent fibers and taste fibers run intimately close to each other. We showed that dental desafferentations in humans (mandibular Vth nerve desafferentation) result in deficits in taste. The topy of the tongue loci where deficits were recorded correspond to the topy of the desafferented teeth (Boucher et al, 2006). As trigeminal fibers terminate in the Nucleus Tractus Solitarius (NTS) amongst taste neurons (Contreras, 1982), we looked for and found functional taste and trigeminal convergences in the rat NTS. Cutting the mandibular nerve branch of the Vth reduced the responses of neurons recorded in the NTS to tastants applied on the tongue (Boucher et al, 2003); on the contrary, stimulating the central part of the mandibular nerve increased them. So, not only lingual trigeminal signals but also mandibular signals contribute to taste and, as a result, somatic stimulation "boosts" taste. Taste and somato-sensory sensitivities cooperate from the very peripheral receptor fields of both types of neurons. In humans, psychophysical evaluations of taste intensity before and after application of Capsaicin (CAP), a somato-sensory stimulus and an agonist of TRPV1, showed inhibition or reinforcement of the perceived intensity of tastants, depending on the compound, the subject and the temporal pattern of application of the stimulus. Hence, the mechanism involved in the modulation of taste by somato-sensory stimuli is not unique but complex. CAP elicits responses in both the Vth nerve and the CT in the hamster. The modulation of CT responses to taste stimuli by CAP was observed provided a response to CAP alone appeared on either the Vth or the CT nerve. Whether this modulation is peripheral or centrally determined remains to be studied. The timing of both nerves responses suggests that one nerve might neurophysiologically "drive" the activation of the other one. However, CT responses to CAP might also result from stimulation of taste cells by capsaicin because channels activated by somato-sensory stimuli exist in taste cells, hence could contribute to CAP modulated taste sensitivity. For example, TRPV1 activated by temperatures above 44°C and by vanilloids including CAP, are present in taste cells where Lyall and col. (2004) showed their potential contribution to NaCl sensitivity. Another argument along the same lines is that in the CT, repeated stimulations induce desensitization of the response to acids only and never to non-acid compounds indicating that there are, apart from taste receptors, mechanisms in taste cells which are similar to those of the Vth nerve fibers to account for desensitization to "somato-sensory" stimuli. Further elements can describe the cooperation between the Vth and the CT. In the hamster, we showed both nerves respond to organic acids relative to the ratio lipophilicity/hydrophobicity of the molecule; these acids elicit responses from lower concentrations in the CT than in the Vth nerve and these lower concentrations correspond to "pleasant acid" in psychophysics whereas concentrations activating also the Vth nerve result in unpleasant acidity leading to painful sensation. Finally, if effects of inhibition or reinforcement on taste responses due to somato-sensory stimuli are well recognized, it is less well known that taste can also affect responses of the somato –sensory system, as we shall see with nerve recordings, behavior in the rat and psychophysics in humans. In conclusion, interactions between taste and somato-sensory sensitivity constitute a growing field of study.

PS24 - TRPM5, a molecular locus for modulation of taste with tastants.

Karel Talavera

KU Leuven, Laboratory of Ion Channel Research, Leuven, Belgium

Ordinary gustatory experiences are determined by multiple interactions between different taste stimuli. The most studied model for these gustatory interactions is the suppression of the responses to sweeteners by the prototype bitter compound quinine. We have previously shown that quinine and quinidine inhibit the taste transduction channel TRPM5, which functions as a calcium-activated nonselective cation

channel involved in the transduction of sweet, bitter, and umami tastes. In addition, we found that these compounds inhibit the gustatory responses of sweet-sensitive gustatory nerves in wild type, but not in Trpm5 knockout mice. We further investigated whether other bitter compounds known to modulate taste perception also affect TRPM5. We found that nicotine inhibits TRPM5 currents with an effective inhibitory concentration of 1.3 mM. This effect may contribute to the inhibitory action of nicotine on gustatory responses in therapeutic and experimental settings, where nicotine is often employed at millimolar concentrations. Taken together, these findings indicate that the inhibition of TRPM5 by bitter compounds constitutes the molecular basis of a novel mechanism of taste interactions, whereby the bitter tastant inhibits directly the sweet transduction pathway. Furthermore, our data imply the existence of a TRPM5-independent pathway for the detection of the bitterness of quinine and nicotine. We also found that TRPM5 currents were unaffected by nicotine's metabolite cotinine, the intensive sweetener saccharin or by the bitter xanthines caffeine, theobromine, and theophylline. This demonstrates that bitter compounds have differential effects on key elements of the sweet taste transduction pathway, suggesting for heterogeneous mechanisms of bitter–sweet taste interactions.

PS25 - Brain mechanisms of taste quality perception.

Kathrin Ohla

German Institute of Human Nutrition Potsdam-Rehbrücke, Nuthetal, Germany

In most species, the sense of taste is key for discriminating potentially nutritious and harmful food constituents and thereby for the acceptance (or rejection) of food. While previous research has focused on how tastants are encoded at the receptor level, it is currently unknown when and where taste quality representations are established in the cortex and whether these representations are used for perceptual decisions. To address this question, we measured spatio-temporal brain responses with electroencephalography (EEG) while human participants assessed and responded to liquid tastants inducing salty, sweet, sour, or bitter sensations. The EEG yielded latency differences between different tastes that were more pronounced when speeded reactions were required. In this case, neuronal response latencies correlated with behavioral responses latencies. Spatial neuronal response patterns, on the other hand, allowed decoding which of the four tastants participants tasted on a given trial. The onset of this prediction coincided with the earliest taste evoked response (at 175 ms) originating from the insula and opercular cortices indicating that quality is among the first attributes of a taste represented in the central gustatory system. These response patterns correlated with perceptual decisions of taste quality: tastes that participants discriminated less accurately also evoked less discriminated brain response patterns. The results therefore provide evidence for a link between taste quality-related decisions and the predictive value of spatial brain response patterns.

PS26 - Taste cell detection of caloric sugars independently of T1rs.

Sunil Sukumaran, Karen K. Yee, Shusuke Iwata, Ramana Kotha, Robert Quezada-Calvillo, Buford L. Nichols, Sankar Mohan, B. Mario Pinto, Noriatsu Shigemura, Yuzo Ninomiya, and Robert F. Margolskee

Monell Chemical Senses Center, Philadelphia, USA

Sugars and non-caloric sweeteners are detected by mammalian taste cells by the heteromeric combination of type 1 taste receptors 2 and 3 (T1r2+T1r3, encoded by Tas1r2 and Tas1r3). This may be the only receptor of non-caloric sweeteners. Yet, Tas1r3 knockout mice can sense many mono- and disaccharides, but not non-caloric sweeteners. Using RT-PCR, qPCR, in situ hybridization and immunohistochemistry, we show that several proteins previously known to be expressed in the intestinal brush border are preferentially co-expressed with T1r3 in taste cells. Among these proteins are alpha glycosidases, glucose transporters (GLUTs), sodium-glucose cotransporter1 (SGLT1) and the ATP-gated (KATP) metabolic sensor K+ channel. Treating the tongue with inhibitors of disaccharidases specifically decreased gustatory nerve responses to disaccharides but not to monosaccharides or to non-caloric sweeteners. Further, treating fungiform taste cells with KATP channel inhibitors decreased potassium currents in these cells, consistent with the function of KATP channels in these cells. We propose that oral alpha glycosidases generate monosaccharides from starch and disaccharides. The monosaccharides are transported into taste cells by GLUTs and SGLT1, in much the same way as in the intestine. Metabolism of these sugars results in elevation of taste cell ATP and consequently, KATP channel closure, membrane depolarization and neurotransmitter and/or neuropeptide release. Thus, the taste cells that respond to sweet compounds likely contain two sweet-sensing pathways: T1r-dependent (i.e. T1r2+T1r3) for detecting sugars and sweeteners, and T1r-independent (i.e. alpha glycosidases, GLUTs, SGLT1, KATP) for detecting caloric sugars.

PS27 - The role of CD36- and GPR120 in response to fatty acids in cultured human taste papillae (HBO) cells.

Mehmet Hakan Ozdener, Selvakumar Subramaniam, Sinju Sundaresan, Omar Sery, Toshihiro Hashimoto, Yoshinori Asakawa, Philippe Besnard, Nada A Abumrad, and Naim A Khan

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Cell cultures are an indispensable tool for basic mechanistic studies, and they are also widely used in applied studies, such as compound screening or therapeutic applications. We recently developed methods for long-term culture of human

taste bud cells. We collect biopsies of cells from human fungiform taste papillae and can maintain them as a primary cell culture for more than a year without loss of molecular and physiological properties. Cultured human fungiform papillae (HBO) cells express proteins involved in taste transduction in vivo, and respond to application of taste stimuli by increases in intracellular calcium. We used cultured human taste bud cells to analyze their sensitivity, breadth of tuning, and effects of taste blockers, and found that these cells retain functional similarity with native taste bud cells and with known taste receptors in heterologously expressed systems. Therefore, the cultured human taste bud cells can be used as a model system for studying mechanisms of taste transduction. Studies in mice suggested that CD36 (CD36 antigen) and GPR120 (G protein coupled receptor 120) are involved in effects of lipids on taste bud cells. However, the role of these candidate fat taste transduction molecules in humans has not been known. Using our proprietary system for long-term culture of human fungiform taste papillae (HBO) cells, we have shown that they respond to fatty acids by increasing intracellular calcium concentration, and that these responses are mediated by CD36 and GPR120. Cultured human fungiform taste bud (HBO) cells co-express CD36, GPR120, and proteins involved in intracellular transduction of taste: a phospholipase PLC-β2 and a G protein α-gustducin. To knockdown the CD36 and GPR120 genes, we transfected the cultured human taste cells with small interfering RNAs against messenger RNAs encoding CD36 and GPR120. The gene knockdown selectively diminished the expression of these proteins in the cultured taste cells, and reduced their intracellular calcium responses to fatty acids. Fatty acid induced signaling transduction mechanisms demonstrated similar to mice via CD36 and GPR120 receptors in HBO cells using number of inhibitor for Protein Tyrosine Kinase and G-Protein Dependent signaling pathway. These data show that similarly to mice, CD36 and GPR120 have non-overlapping roles in orogustatory perception of dietary lipids by humans. Previously, we have shown that the cultured human taste cells are a useful tool to study mechanisms of the main five taste qualities and can be used for screening taste compounds associated with these taste qualities. The current study shows that these cells are also useful for studying mechanisms of fat taste and can be used for developing new fat substitutes.

PARALLEL SYMPOSIUM VI: OLFACTORY BULB INTERNEURON CIRCUITS: FROM PROCESSING TO PLASTICITY.

PS28 - Revealing maps and mechanisms of interneuron circuit formation.

Benjamin Arenkiel

Baylor College of Medicine, Department of Molecular and Human Genetics, Houston, USA

Elucidating patterns of functional synaptic connectivity, and deciphering mechanisms of how plasticity influences such connectivity is essential towards understanding brain function. In the mouse olfactory bulb, mitral/tufted cells make reciprocal connections with local inhibitory interneurons, including granule cells and external plexiform layer (EPL) interneurons. Our current understanding of the functional connectivity between these cell types, network responses, as well as their experience-dependent plasticity remains incomplete. We have begun to map connections between mitral cells and these interneuron subtypes in a cell type-specific manner, and found that EPL interneurons receive broader and stronger mitral cell input than granule cells, and that these interneuron types exhibit distinct patterns of local connectivity with mitral cells. Using an olfactory associative learning task, we further describe how these circuits displayed differential propensity for experience-dependent plasticity. Whereas the reciprocal connectivity between mitral cells and EPL interneurons was non-plastic, the connections between granule cells and mitral cells were dynamic and adaptive. Using in vivo calcium imaging of odor responses, we also compared functional responses of genetically defined populations of both maturing and established granule cells. We found that in contrast to the activity-dependent refinement and pruning observed for excitatory maps, inhibitory sensory maps become broader with interneuron maturation. Together, these data show that different interneuron types form distinct connectivity maps and modes of experience-dependent plasticity in the olfactory bulb, which may reflect their unique functional roles in information processing, while also highlighting the differences between inhibitory and previously described excitatory maps.

PS29 - Plasticity and precision in olfactory bulb circuits.

Leonardo Belluscio

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The anatomical organization of the olfactory bulb is remarkably precise given its capacity for plasticity and regeneration. The presence of olfactory bulb maps accentuates that precision and provide a consistent framework from which to measure any changes in connectivity. We found that three broad factors: plasticity, activity and regeneration each play an important part in the formation and maintenance of olfactory bulb circuitry and are trying to understand the role of adult born neurons in this process. Using a combination of anatomical, pharmacological and imaging techniques we show that plasticity, activity and regeneration all work interdependently and that the regenerating interneurons play a key function in maintaining the stability and organization of the olfactory bulb network.

PS30 - Plasticity of Inhibitory circuits in accessory olfactory bulb after social experience.

Ian Davidson

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Abstract pending.

PS31 - Interneuron shaping of MC/TC responses in awake, behaving mice.

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Inhibitory circuits are a hallmark of computation in the brain. In the olfactory system, we have recently shown that inhibition in the mouse olfactory bulb (OB) is involved in odour fine discrimination behaviour. Optogenetic silencing experiments in anaesthetized and passive awake mice have suggested that distinct interneuron circuits fulfil specific roles in the OB, namely that superficial interneurons shape slow temporal features while deep interneurons, most notably including granule cells (GCs), orchestrate activity across the OB on faster timescales. Surprisingly, silencing GCs in the anaesthetized preparation had essentially no impact on baseline activity of projection neurons both in anaesthetized and awake animals. Moreover, GC themselves were virtually silent (largely less than 1 Hz baseline firing) in both anaesthetized and passive awake mice. Here I will discuss these results and compare the contribution of inhibitory circuits in anaesthetized, passive awake and behaving mice using cell-specific optogenetic silencing, imaging and whole-cell recordings in head-fixed preparations.

PS32 - Activation of the mouse OR37 subsystem coincides with a reduction of novel environment-induced activity within the paraventricular nucleus of the hypothalamus.

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Within the main olfactory system of mammals, a unique subsystem exists comprised of sensory neurons expressing odorant receptors of the OR37 subfamily. These receptors are exclusive for mammals and highly conserved across species. The mouse OR37 receptor subtypes A, B and C were shown to be activated by the long-chain aliphatic aldehydes penta-, hexa- and heptadecanal, respectively. The search for biological sources of these compounds now shows that bodily secretions from conspecifics activate the OR37A, B and C glomerulus. At the same time the activity of cells in a target region of projection neurons from OR37 glomeruli, the paraventricular nucleus of the hypothalamus (PVN), is reduced compared to controls (clean test box). The large number of activated cells in the PVN of mice that are placed into a clean test box are corticotropin-releasing hormone cells, indicating an induction of the stress axis due to the novel environment. The much lower number of activated cells of mice in a box enriched with bodily secretions from conspecifics indicates a reduced stress response. Since bodily secretions from conspecifics activate the OR37 system and simultaneously reduce stress-induced activation of the PVN, it was tested whether the ligands for OR37 receptors can induce this effect. Indeed, a similarly reduced activity in the PVN is found in mice kept in a clean test box and exposed to a mixture of the OR37 ligands delivered via an air stream. These data indicate that the OR37 system may play a role in mediating a phenomenon called social buffering.

Funding acknowledgement: This work was supported by the Deutsche Forschungsgemeinschaft (SPP1392).

PS33 - Function of contra-lateral interactions between olfactory bulbs.

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Similar to vision or audition, the peripheral olfactory system consists of a pair of sensory organs. Olfactory information coming from a given nostril is sent to the ipsilateral olfactory bulb and then transmitted to the olfactory cortices. This suggests an independent and somehow redundant encoding of olfactory information by two parallel olfactory channels. Previous anatomical studies suggest that two parallel olfactory channels can interact at least through contralateral top-down projections from the olfactory cortex. However the extent of these contralateral interactions between parallel olfactory channels and their function in odor processing is not studied in detail. In order to identify the anatomical basis of connections between two olfactory bulbs we first used dye electroporation. Our results showed that the two olfactory bulbs are strongly connected in addition to the contralateral projections from the cortical areas. We observed that while top-down projections from the olfactory cortices are rather diffuse and mostly terminating at the inhibitory granule cells layer, the direct connections between olfactory bulbs are layer specific and mostly terminating at the mitral-cell/glomerular layer of the olfactory bulb. Using targeted dye electroporation in genetically identified glomeruli, we showed that these direct connections between olfactory bulbs are topographically organized, where sister glomeruli in contralateral olfactory bulbs are strongly linked with direct mitral cell projections. In order to study the function of these direct and indirect interactions between two parallel olfactory pathways, we used a combination of intra-cellular recordings, electrical micro-simulation and two-photon calcium imaging. Our results showed that information coming from one olfactory bulb can significantly alter odor responses in the contralateral olfactory bulb, eliciting a balance of excitation and inhibition. Interestingly, low intensity contralateral stimulations elicit mostly excitation whereas high intensity contralateral stimulations also elicit inhibition, suggesting a role for contralateral connections in regulating gain control.

YOUNG SCIENTIST SYMPOSIUM: NEXT-GENERATION SCIENTISTS IN SMELL AND TASTE RESEARCH.

YS1 - Muscarinic receptors 2 and 5 mediate a negative autocrine feedback mechanism in urethral brush cells activated by bitter stimuli.

Klaus Deckmann[1], Gabriela Krasteva-Christ[2], Amir Rafiq[1], Paul Scholz[3], Sabrina Baumgart[3], Thomas Bschleipfer[4], and Wolfgang Kummer[1]

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Urethral brush cells (UBC) are cholinergic chemosensory cells functionally expressing the canonical bitter and umami taste transduction signaling cascade (α-gustducin, PLCβ2, TRPM5). Recently, we described them as sentinels initiating local or reflexive protective responses at the entrance to the urogenital tract, communicating via acetylcholine (ACh) release. They also respond to ACh by themselves in that the bitter (denatonium) evoked increase in [Ca2+]i is enhanced in presence of a muscarinic/nicotinic blocker cocktail. Consecutively, an autocrine feedback mechanism seems to be involved in the regulation of the system sensitivity. Expression levels of muscarinic receptors (MR) were determined with RT-PCR, qPCR, and Deep Sequencing of murine UBC. Confocal laser scanning microscopy based intracellular [Ca2+]-imaging was used to record the specific response of UBC isolated from MR knockout animals after stimulation with denatonium without and in presence of a muscarinic/nicotinic blocker cocktail. In expression studies, mRNAs coding for all MR were detected, although only those coding for subtypes 2, 3, and 4 were consistently found. Inconsistent detection of M1R and M5R might be due to expression below the detection threshold. Comparable to control animals, M1R, M3R and M4R knockout animals showed an increase in intracellular calcium concentration in response to denatonium. In UBC isolated from M2/3R double knockouts or M5R knockouts, however, the inhibitors did not enhance the bitter response. Our results show an autocrine, cholinergic negative feedback loop on activated UBC mediated via M2R and M5R.

YS1 - Metamorphotic remodeling of the olfactory organ of the African clawed frog Xenopus laevis.

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The amphibian olfactory system undergoes massive remodeling during metamorphosis. The transition from aquatic olfaction in larvae to semi-aquatic or airborne olfaction in adults requires anatomical, cellular and molecular modifications. These changes are particularly pronounced in Pipidae, whose adults have secondarily adapted to an aquatic lifestyle. In the fully aquatic larvae of Xenopus laevis, the main olfactory epithelium specialized for sensing water-borne odorous substances lines the principal olfactory cavity (PC) while a separate olfactory epithelium lies in the vomeronasal organ (VNO). During metamorphosis, the epithelium of the PC is rearranged into the adult 'air nose', while a new olfactory epithelium, the adult 'water nose', forms in the emerging middle cavity (MC). Here we performed a stage by stage investigation of the anatomical changes of the Xenopus olfactory organ during metamorphosis. We quantified cell death in all olfactory epithelia and found massive cell death in the PC and the VNO suggesting that the majority of larval sensory neurons is replaced during metamorphosis in both sensory epithelia. The moderate cell death in the MC shows that during the formation of this epithelium some cells are sorted out. Our results show that during MC formation some supporting cells, but not sensory neurons, are relocated from the PC to the MC and that they are eventually eliminated during metamorphosis. Together our findings illustrate the structural and cellular changes of the Xenopus olfactory organ during metamorphosis.

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YS3 - Hierarchical deconstruction of olfactory sensory neurons: from whole organ to single-cell RNA-seq.

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The mouse olfactory mucosa is a complex chemosensory tissue composed of multiple cell types, neuronal and non-neuronal. We have here applied RNA-seq hierarchically, starting with crude tissue samples dissected from the nose, proceeding to flow-cytometrically sorted pools of mature olfactory sensory neurons (OSNs), and finally arriving at single mature OSNs. We show that 98.9% of intact olfactory receptor (OR) genes are expressed in mature OSNs, and uncover a hitherto unknown bipartition among mature OSNs. We find that 19

of 21 single mature OSNs each express a single intact OR gene abundantly, consistent with the one neuron-one receptor rule. Monoallelic expression of this abundantly expressed OR gene is extremely tight. The remaining two single mature OSNs appear to be examples of type B Trpc2+ cells in the main olfactory epithelium, which we here establish as a neuronal cell type that is fundamentally distinct from canonical OSNs.

YS4 - The sniffing brain.

Ofer Perl, Tali Weiss, Liron Pinchover, Lavi Secundo, Nofar Mor, Roni Kahana, Lee Sela, and Noam Sobel

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The primary role of respiration is regulation of homeostasis to maintain life. However, the respiratory cycle also plays an important role in sensory perception. Most terrestrial mammals rely on chemosensory sampling of the environment through rhythmic nasal inhalation, namely sniffing. Olfaction, however, is not the only sense orchestrated by the respiratory cycle, and several mechanisms of mammalian sensory acquisition act in phase with ongoing respiration. Whether humans exhibit such respiratory-driven modulation of sensing or mentation remains unknown. With this in mind, we set out to ask whether respiration mode (inhalation or exhalation) affects performance of simultaneously conducted cognitive tasks. First, we asked whether humans subliminally opt to engage in cognitive tasks during a specific respiratory phase. To answer this we monitored nasal respiration of subjects as they engaged in a battery of cognitive tasks where task-onset was self-initiated. We found that subjects subliminally self-initiated tasks at particular phases of the respiratory cycle (Circular m-test: $p < 0.05$ in 21 out of 26 subjects in a verbal task, 22 out of 24 subjects in a mathematical task, and 26 out of 29 subjects in a spatial task). Having established a link between respiratory phase and cognitive performance, we then used the subjects' own respiration to trigger a verbal task (lexical decision) time-locked with inhale and exhale onset. Reaction time for "non-word" trials was lower for words presented during nasal inhalation compared with exhalation. This modulatory effect however, was not evident in oral respiration, suggesting a unique role for nasal inhalation in cognitive processing (ANOVA nasal/oral X word/non-word X inhale/exhale- $F(1,39) = 5.16$, $p < 0.03$). Finally, we replicated this experiment within the fMRI environment to explore the neural correlates associated with these effects. Results from this part of the study will be presented at the meeting.Taken together, we suggest that human brain function fluctuates between two modes of activation driven by the respiratory cycle, with inhalation being associated with improved acquisition and processing of information.

YS5 - From Mouse to Man: bacterial signal peptides provide a new mechanism for sensing pathogens.

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There is growing evidence that the sense of smell contributes to the defense against pathogens. However, the underlying physiological and molecular mechanisms remain unclear. Our goal is to identify the molecular machinery involved in odor-mediated defense against pathogens by combining high-throughput receptor pharmacology, cellular physiology and behavioral tests. Here, we propose that recognition of bacterial signal peptides by mouse and human formyl peptide receptors (FPRs) represents a novel mechanism for the detection of pathogens by both the immune- and olfactory systems. The recent identification of members of the FPR G-protein-coupled receptor family in the vomeronasal organ (VNO), a key organ for odor-guided behaviors, offers an exciting opportunity to study the molecular basis of olfactory pathogen sensing. The well-documented contribution of FPRs for human immune defense suggests that vomeronasal FPRs in mice may also be used for olfactory pathogen sensing and/or avoidance of sick conspecifics. During the past years, we established high-throughput assays that permit the functional in vitro analysis of FPR receptors and are suitable to examine primary cells such as sensory neurons and leucocytes. Systematic structure-function analyses of 20 FPRs from humans, mice and four other species with a large agonist panel showed that the pharmacological profile of VNO-FPRs differs considerably from that of structurally related FPRs in the immune system (Bufe et al., JBC 2012). During the course of these studies, we identified a set of agonist properties, common to both VNO and immune FPRs that show typical features of a pathogen-associated molecular pattern (Bufe et al., JBC 2015). Guided by these results, we discovered that bacterial signal peptides, normally used to translocate proteins across cytoplasmic membranes, are a vast natural family for FPR activators that trigger human and mouse innate immune responses. With at least 175,542 predicted sequences, bacterial signal peptides represent one of most complex classes of G-protein-coupled receptor agonists currently known. Intriguingly, bacterial signal peptides are capable of activating both FPRs from the immune system and the VNO. Although some human and mouse immune FPRs seem to be broadly activated by signal peptides, the mouse vomeronasal receptor mFpr-rs1 and its human sequence orthologue hFPR3 are far more selective. Possibly, FPR1 and FPR2 function as more general sensors of bacteria, whereas hFPR3 and mFpr-rs1 focus on the detection of specific sets of bacterial pathogens. To demonstrate conclusively that bacterial signal peptides are

involved in odor-mediated defense against pathogens, cellular and behavioral analyses in mice using these peptides will be required.

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YS6 - Feedforward excitation entrains oscillatory activity in a subpopulation of mitral cells in the accessory olfactory bulb.

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The accessory olfactory system is a key component in rodent conspecific chemical communication. However, coding strategies within the involved brain areas - the accessory olfactory bulb (AOB), the 'vomeronasal' amygdala and the hypothalamus - are poorly understood. In the AOB, the first stage of information processing in the mouse vomeronasal pathway, mitral cells (MCs) - the AOB's projection neurons - receive sensory input from vomeronasal neurons. This sensory information is processed in the AOB network before it is relayed to third-order nuclei. A subpopulation of MCs exhibits slow oscillatory discharge that persists upon pharmacological inhibition of fast synaptic transmission. Here, we identify an excitatory circuit within the AOB network that entrains oscillatory activity in a second MC subpopulation. Using patch clamp recordings from MCs in acute AOB tissue slices, we investigate the mechanisms underlying oscillatory entrainment. Entrained MCs display periodically increased excitatory synaptic input that correlates with their respective rhythmic discharge patterns. Block of fast glutamatergic synaptic transmission reveals that entrainment depends on an intact glutamatergic network. Ongoing experiments aim to identify the detailed mechanisms of MC entrainment and the role of slow rhythmic activity in AOB information processing.

Funding Acknowledgement: This work was supported by the Deutsche Forschungsgemeinschaft SPP 1392: "Integrative Analysis of Olfaction"

YS7 - Contribution of TMEM16A to the calcium-activated chloride current in mouse vomeronasal sensory neurons.

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The vomeronasal organ is involved in the detection of pheromones. The binding of pheromones to receptors in microvilli of vomeronasal sensory neurons (VSNs) activates a phospholipase C signaling cascade producing the entry of Na+ and Ca2+ through TRPC2 channels. In this study, we have used patch-clamp recordings to provide a characterization of currents activated by Ca2+ in isolated mouse VSNs. In whole-cell recordings, we found that 70% of VSNs have a Ca2+-activated current Cl^- current. Inside-out patches from dendritic knobs confirmed the presence of Ca2+-activated Cl^- channels in the knobs and/or microvilli of mouse VSNs. Comparing our results with the properties of heterologous expressed TMEM16A and TMEM16B Ca2+-activated chloride channels, which are co-expressed in mature VSNs, we found a closer similarity to those of TMEM16A. Taking advantage of the expression of the olfactory marker protein (OMP) in mature VSNs, we selectively knocked out TMEM16A in these neurons, by crossing floxed TMEM16Afl/fl mice with mice expressing Cre recombinase under the control of the OMP promoter. The specific ablation of TMEM16A in mature VSNs was confirmed by immunohistochemistry; while the expression of other membrane proteins present in the microvilli, such as TRPC2 and TMEM16B, remained unaltered. Whole-cell recordings from TMEM16A conditional knockout mice showed that Ca2+ activated currents were abolished, while voltage-gated inward currents were present in VSNs. These results demonstrate that the TMEM16A protein is expressed in mature VSNs and is an essential component of Ca2+-activated Cl^- currents in mouse VSNs.

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YS8 - Unzipped mediates Drosophila mushroom body development through neuron-glia interactions.

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Drosophila mushroom bodies (MB) are required for complex behaviors including learning, memory, and odor-driven attraction/repulsion. The MB consists of three types of neurons, which are generated from the same neuroblasts in a temporal manner. Each neuron type has it's own projection pattern and it is known that early born neurons guide axons of late born neurons. It has been known for a long time that glia have very important roles in axon guidance. However, specific examples for their role in axon guidance are very limited. Even for the development of MBs, which has been investigated in detail for years, the role of glia in this process is unknown. Unzipped has been identified as a novel cell adhesion molecule that is mostly expressed in glial

cells. Here we showed that uzip regulates MB development through neuron-neuron and neuron-glia interactions and expression by both cell populations is required for proper MB development.

YS9 - Electrical stimulation of the human olfactory mucosa fails to generate olfactory perception yet alters activity in primary olfactory cortex: a novel path to human brain stimulation.

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The mammalian olfactory system is in contact with the external environment through bipolar sensory neurons with nerve endings in the nasal mucosa. Efforts to artificially stimulate these nerve endings using electrical currents applied in the human nose have yielded mixed results. Here we used endoscopically guided electrode placement to electrically stimulate these targets in 46 healthy human subjects who participated in 139 stimulation sessions. Despite application of varied electrical stimulation parameters, we never once succeeded to artificially induce the perception of odor. In turn, stimulation drove an assortment of non-olfactory sensations, including nasal itching, tingling, cooling, and visual flashes of light (phosphines). Furthermore, sub-detection threshold electrical stimulation applied concurrently with odor drove a small but significant reduction in the perceptual pleasantness associated with the odor ($T(15) = 3.3$, $p < 0.005$). Finally, we tested whether such stimulation is reflected in patterns of brain activation as measured with functional magnetic resonance imaging. We tested 20 subjects, each scanned twice in a 3-Tesla Siemens MRI magnet, once after electrical stimulation and once after sham stimulation. Subjects could not tell which experiment was sham and which was electrical stimulation (Wilcoxon signed rank test $Z = 26$, $p = 0.33$). Nevertheless, sub-detection-threshold unilateral electrical stimulation of the nasal mucosa drove decorrelation of neural activity between left and right primary olfactory cortex ($F(1,18) = 15$ $p = 0.001$). Taken together, we conclude that electrical stimulation of the nasal mucosa fails to generate olfactory perception despite downstream modulation of neural activity. This failure to induce olfactory perception has implications for the interpretation of artificial stimulation studies in olfaction. Although those studies may be highly informative, even if accompanied by selective activation of downstream olfactory targets, one must take caution in assuming that this necessarily implies induction of odor perception. Furthermore, the results of selectively altered brain activity may imply the potential for a novel minimally invasive path to deep human brain stimulation.

YS10 - Multiplex assessment of the positions of odorant receptor-specific glomeruli in mouse olfactory bulb by serial two-photon tomography.

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In the mouse, axons of olfactory sensory neurons (OSNs) that express the same odorant receptor (OR) gene coalesce into one or a few glomeruli in each olfactory bulb. The positions of OR-specific glomeruli have traditionally been described as stereotyped. Here we have assessed the positions of OR-specific glomeruli using serial two-photon tomography, a novel automated method for whole-organ fluorescence imaging that integrates two-photon microscopy with serial microtome sectioning in a fully unsupervised manner. Our strategy is multiplexed: by repeated crossing we generated two strains of mice with gene-targeted mutations at four or five OR loci. We thus analyzed a total of six OR-specific glomeruli: MOR23 (Olfr16), mOR37A (Olfr155), P2 (Olfr17), MOR256-17 (Olfr15), MOR28 (Olfr1507), and M72 (Olfr160). Glomerular imaging relied on the intrinsic fluorescence of GFP or dsRed, or on whole-mount immunofluorescence with antibodies against GFP or β-galactosidase using the iDISCO protocol. The high-resolution 3D-reconstructed datasets were segmented to identify the fluorescent glomeruli, and to determine their positional variability between the bulbs of one mouse (intra-individual) and among the bulbs of different mice (inter-individual). We discover a substantial degree of variability in glomerular positions. Importantly, this variability depends on the OR: for instance, the medial MOR28 glomerular domain is spread over a surface area in the bulb that is an order of magnitude larger than that of the medial MOR23 glomerular domain. Our results quantify the level of precision that is delivered by the mechanisms of OSN axon wiring, differentially for the various OSN populations expressing distinct OR genes.

POSTER CONTRIBUTIONS

P1 - Odors in human cerumen (earwax).

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Human body odor, which is made of volatile organic compounds (VOCs), contains meaningful information on physiological and mental state. Although most investigations into body odor examine axillary odorants in sweat, humans secrete several additional potential odor carriers. One such source is cerumen (earwax). Cerumen is produced by sebaceous glands and ceruminous glands, which are specialized

sweat glands located in the external ear canal. The function of cerumen secretion is not fully understood. It is hypothesized to take part in the moistening, cleaning and humidification of the ear canal. There are also inconclusive data regarding its antimicrobial function. Notably, polymorphism in cerumen phenotypes results from polymorphism in a gene central to human odor production (ABCC11). With this in mind, we set out to establish a VOCs profile for human cerumen secreted in various physiological and mental conditions. We obtained cerumen from 11 healthy individuals (6 F, age = 37.8 ± 17.4) by way of suction from the ear canal. We subjected cerumen headspace to VOC analysis using gas chromatography mass spectrometry (GC-MS). In brief, we incubated cerumen samples with PDMS-covered stir bars (Gerstel Twister) at 37ºc, overnight. Then Twister was desorbed in a Thermal desorption Unit (TDU, Gerstel GmbH, Germany). A recent comprehensive study of cerumen VOCs identified several compounds (Prokop-Prigge et al., 2014). Here, we identified four additional VOCs that were not describe previously in the literature, as far as we know. Γ-carpolactone, a known female dermestid beetle (Trogoderma glabrum) pheromone, which was also demonstrated to stimulate the growth and activity of the biocontrol agent Rhodococcus erythropolis. This component was previously detected in human urine and the polar fraction of the blood. The second was 2-undecanone, which was demonstrated to act as an insect and animal repellent. Finally, we also identified δ-caprolactone and 2-hexanone in this ceruminous medium. We are continuing to collect data in order to determine whether these compounds are genuinely secreted by humans or are the result of contamination (e.g., cosmetics), and further estimate the variability of these secretions as a function of physiological and mental state.

P2 - TRPV1 contributes to acrolein-induced toxicity.

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Acrolein is a toxic and highly reactive unsaturated aldehyde, often found in cigarette smoke and vehicle exhaust gases. Likewise, acrolein derived from cyclophosphamide-treated patients constitutes the major culprit of bladder irritation during chemotherapy in cancer patient. Although, initially, its toxicity and inflammatory properties have been related to the activation of the transient receptor potential A1 (TRPA1) in nociceptive neurons, recent evidences in TRPA1-deficient mice suggested that other receptors may also play a role in acrolein-induced toxicity. Here we show that, besides activation of TRPA1, acrolein evokes the activation of TRPV1 channels. Ratiometric calcium measurements and patch-clamp experiments suggested that unlike TRPA1 that desensitizes immediately after activation, acrolein-induced activation of TRPV1 is prolonged in time. Furthermore, we identify the N-terminal amino acid residue C157 as key for acrolein-induced TRPV1 activation. Taken together, our results reveal a mechanism underlying the major role of TRPV1 as mediator for the acrolein-induced toxicity, unveiling TRPV1 as a potential therapeutic target in a wide spectrum of noxious conditions, from exposure to smoke to cancer treatment.

P3 - Body odor conveys information about trustworthiness.

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Human social chemosignaling serves an important function in human communication. However, the exact nature of the information conveyed in this form of communication remains largely unknown. Here we test the hypothesis that a person's body odor carries information about their trustworthiness. We used the trust game, a commonly applied behavioral economics paradigm, to measure trustworthiness. 57 participants (25F, mean age 26 ± 3.3 years) played the role of the trustee in the trust game, and the average amount of money that was sent back to the investor served as an index of their trustworthiness. Subsequently, 10 male subjects (mean age 28.4 ± 2.3 years) spanning the trustworthiness distribution were asked to serve as body odor donors and slept with a provided t-shirt for two consecutive nights. 35 subjects (21F, mean age 25.2 ± 3.1 years) were asked to rate these body odors for trustworthiness, pleasantness and intensity. Analysis of the rankings revealed two significant correlations: In male raters, there was a correlation between actual trustworthiness and perceived trustworthiness (spearman's rho=0.63, $p<0.05$) and in female raters there was a correlation between actual trustworthiness and perceived pleasantness (spearman's rho=0.71, $p<0.05$). Comparing the body odors of the two subjects who were ranked on average as least trustworthy and most trustworthy among male raters revealed a nearly significant difference in trustworthiness rankings ($p=0.08$, Wilcoxon signed-rank test), while the odors did not differ significantly in pleasantness and intensity ($p=0.62$ and $p=0.12$ respectively, Wilcoxon signed-rank test). The same comparisons were made between the two subjects which female raters ranked as least and most trustworthy, and their odors were found to be significantly different in trustworthiness ($p<0.01$, Wilcoxon signed-rank test) and pleasantness ($p<0.005$, Wilcoxon signed-rank test), with the lowest-trustworthiness odor being perceived as less pleasant than the highest-trustworthiness odor. No significant difference was found between the intensity rankings of the two odors ($p=0.37$, Wilcoxon signed-rank test). Taken together, these results imply that body odor may convey

meaningful information about trustworthiness, and that this information is processed differently in males and females.

P4 - Pinch of salt: The influence of taste on (orthonasal) odor perception.

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Odor-taste combinations constitute a salient feature of the foods we consume daily. Whether these occur naturally (e.g., sweet grapes) or are the product of our culture (e.g., salted chocolate) certainly shapes our perception of odor-taste congruence. Despite the high ecological value of odor-taste interactions, the actual influence of tastants on odor perception has so far received little attention. We showed previously that participants could evaluate the proportion of the constituents in binary odor mixtures with high accuracy. We therefore set out to explore the influence of the presence of a tastant on the experienced composition of a binary odor mixture and its constituents. We hypothesized that the presence of a taste would influence the experienced odor compared to the absence of taste and that this effect would be modulated by the degree of odor-taste congruence. The stimuli consisted of nine odor-taste pairs based on either of two tastants (sour and savory) or water and either of three odorants (orange, chicken and a perceptual half-half mixture of the two). Participants rated the dominant tone of each odorant presented with a tastant or with water using a visual analogue scale with the labels "orange" and "chicken" at the extremes and the intensity and pleasantness of the odors and the congruence of the pairs using visual analogue scales. Overall, odors were more intense and less pleasant when paired with a taste compared to water irrespective of congruence. The perceived odor composition was influenced by and in the direction of the incongruent taste for the pure odorants only. These results show that oral tastes influence orthonasal odor perception at the sensory and hedonic levels and bear implications for food preference particularly with regard to composition.

P5 - Comparing the effects of isocaloric oral and i.v. application of nutrient solutions on olfactory functioning.

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The relationship between food intake and olfactory functioning is commonly investigated for the effects of oral, rather than for parenteral administered nutrients. The primary aim of our research was to investigate the influence of isocaloric oral and i.v. application of a nutrient solution on olfactory sensitivity, discrimination, identification and on intensity and hedonic ratings. Conducting two studies we involved 30 participants and administered an isocaloric application of nutrient solutions orally (Study 1) or intravenously (Study 2).In Study 1, 10 healthy men ingested a nutrient mix solution (carbohydrate, fat, protein) in randomized order on one day as a bolus on the other day sustained over the study day. In Study 2, 20 healthy men were tested on 4 different study days. Each day participants received one of three isocaloric (carbohydrate or fat or protein) or a placebo (NaCl) infusion in randomized order. Each solution was administered continuously during the study day. We tested olfactory functioning by means of the "Sniffin´ Sticks" test battery comprising a threshold (n-butanol), a discrimination and an identification test. Participants rated odor intensity and hedonics for the odorants of the identification test by means of analog rating scales. Our studies revealed that method of application (oral bolus or oral sustained release) has a significant influence on hedonic ratings; statistical trends were observed for odor discrimination and intensity ratings. Odor identification significantly improved when comparing hunger state and satiated state of the oral sustained release condition. But this effect was not observed for the oral bolus application condition. Isocaloric fat infusion significantly decreased the olfactory threshold of n-butanol compared to summated data of carbohydrate, protein and placebo. We found no significant differences between all nutrient solutions in odor discrimination, identification and in intensity or hedonic ratings. Summarizing our results motivate an ongoing study of our laboratory comparing the effects of i.v. versus oral administration of three isocaloric nutrient solutions (carbohydrate or fat or protein versus placebo).

Funding acknowledgement: Part of this study was undertaken within the Neurotrition Project, which is supported by the FAU Emerging Fields Initiative.

P6 - The cilia of rat olfactory sensory neurons incorporate glucose and may take it from the mucus to use as complementary energy source for odor transduction.

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Olfactory cilia (~60 μm long, 0.2 μm diameter) project from the apical knob of the single dendrite of olfactory sensory neurons (OSNs). Odorants binding to G-protein coupled receptors trigger a cAMP-cascade in the cilia that leads to the opening of Ca2+-conductive cyclic nucleotide-gated channels (CNGs); Ca2+ itself activates Cl- channels. Both channels generate a depolarizing receptor potential. The cilia require ATP for the activity of the adenylyl cyclase, ATPases and kinases. The mitochondria in the knob are the

closest to the cilia, which lack any inner membranes. Slow ATP diffusion from the knob and limited basal ATP availability in the cilia suggest the possible existence of additional ATP sources to sustain transduction during periods of intense odor stimulation. Immunohistochemistry previously revealed glucose transporters in the olfactory epithelium ciliary layer, populated with OSNs cilia and supporting cells (SCs) microvilli (Nuñez-Parra et al, Chem Senses, 2011). We hypothesized that glucose is released by SCs to the mucus and incorporated by the cilia to produce ATP by glycolysis. We detected the presence of glucose in the mucus covering the epithelia with a colorimetric assay. By immunocytochemistry on dissociated cells we confirmed the presence of the glucose transporter GLU-3 in OSN both in OSN cilia and SC microvilli. With a fluorescent glucose analog (2-NBDG), we observed that the cilia incorporated this sugar from the mucus. Glycolytic enzymes are present in ciliary membranes, as revealed by immunoblotting. Glycolysis as well as oxidative phosphorylation inhibitors partly abolished odor responses in field recordings from the olfactory epithelium and suction pipette recordings from isolated OSNs in the presence of external glucose (0.2 mM). OSNs underwent fatigue upon glucose removal from the extracellular solution. These results support the notion that the cilia take from the mucus glucose released by SCs and process it by glycolysis to supply ATP for chemotransduction, in addition to ATP supplied by the knob.

P7 - Using Odor to Target Local Reactivation of Memory in Sleep.

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Sleep promotes memory consolidation in a process that is posited to involve specific reactivation of recent traces. This reactivation is concurrent with sleep spindles. Reactivation is considered to embody memory transference between the hippocampus and the neocortex, culminating in neocortical long-term memory representations. Presentation of the learning context during NREM sleep has been reported to reactivate the relevant memory and hence promote its strengthening (Diekelman & Born 2010). It has been further suggested that sleep related neuronal patterns, including thalamocortical spindles, function locally in cortical networks that encode the specific memory being processed. With this in mind, we set out to test the hypothesis that cuing targeted at specific brain areas will reactivate specific memories. We set out to perform a memory reactivation experiment using contextual cues to a learned task in a targeted brain area. Odor was selected as the learning context since olfactory stimuli can be presented to sleeping participants without waking them. The participants, whose gaze is focused on the center of a computer screen, are required to remember a word-screen location association, in which half of the locations corresponded to the right visual field and half to the left visual field. The odor (rose) is present throughout training. The learning session is followed by sleep. During NREM sleep, the contextual odor cues are presented only to a single nostril for each participant. Due to the ipsilateral nature of the subcortical olfactory system, and the reduced effective cortical interhemispheral connectivity at sleep, we expect the cues to reach only the hemisphere ipsilateral to the cued nostril, and to augment only the memory of the hemisphere-related screen locations, contralateral to the cued nostril. We also posit to have more powerful spindles in the cued-side hemisphere, reflecting the reactivation process. The memory task was so far tested on 29 subjects and the level of difficulty was fitted to be 60% success before sleep (with chance level being 12.5%). No significant difference in memory between left and right screen locations was found before reactivation. In addition, eye tracking showed participants has no difficulty in keeping their gaze on the fixation point while following the movement of the words. We will report the results of applying this task and unilateral odor exposure in sleeping participants.

P8 - Divided and combined cognition of odor emotionality.

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Different opinions exist about the sequence regarding how the brain processes perceptual features of olfactory input. The two main approaches are the valence-centered approach and the object-centered approach. The valence-centered approach suggests that odor valence is processed before more information about the odor is retrieved. In other words odor names, source and experience with the odor are reconstructed through the specific valence. The object-centered approach on the other hand implies that first an odor object, meaning the perceptual memory of an odor, is processed which then triggers the affective system. In this study, both approaches are challenged by the factor odor emotionality. It is hypothesized that the sequence of odor processing is influenced by odor valence. Moreover, fMRI-Data should give an impression of involved neural substrates in valence and odor object processing. 19 subjects (12 male and 7 female) participated in the current fMRI-study. A MRI compatible multichannel (24 channels) olfactometer was used for presenting many odors in fast intervals. 8 different odors (4 negative and 4 positive) were evaluated in a choice respond time paradigm of 3 blocks (valence: Is the odor pleasant/unpleasant?: Yes or No, object: Is the odor garlic: Yes or no and detection: Is the odor present: Yes or No). Behavioral results showed that negative odors are processed faster in the valence block than in the object block. For positive odors

no significant difference was found between the valence and object block. The activation of the amygdala, a core brain region for emotion processing and emotional memory in addition to piriform cortex activation for odor object and odor valence evaluation points to a more complex construct of an odor object including remembered source information and experience as well as odor emotionality. The behavioral results suggest that negative odors are processed differently than positive odors. Moreover, fMRI data give the impression of a combined valence and object processing. Thus, the evolutionary component of an odor and its genetically anchorage could be discussed that splits the sequence of both approaches into a parallel processing for positive odors, probably in form of an odor object consisting of the perceptual memory including valence as an additional factor and into a direct processing of valence of negative odors to accelerate the response to the external world.

P9 - Olfactory imprinting and related cellular activity in the larval zebrafish olfactory system.

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Imprinting is a learning process during early development which leads to irreversible changes in behavior. Based on behavioral experiments, zebrafish larvae were shown to imprint on olfactory and visual cues of their kin during a 24h time window from day 6 to day 7 post fertilization (Gerlach et al., 2008). Furthermore, zebrafish imprinting was revealed to depend on MHC class II related signals and only larvae that share MHC class II alleles can imprint on each other (Hinz et al., 2012). In contrast to most land vertebrates, the teleostean (e.g., zebrafish) olfactory system lacks a vomeronasal organ and consists only of a paired main olfactory epithelium (MOE) which displays four types of olfactory sensory neurons (OSNs). These perceive olfactory stimuli and mediate odor information into the olfactory bulb, the first central nervous station for odor processing. Similar to mammals, ciliated OSNs express olfactory receptors of the OR and TAAR-families, whereas microvillous OSNs express receptors of the V1R and V2R gene family (Saraiva and Korsching, 2007; Hussain et al., 2009; Oka et al., 2011) Additionally, teleostean olfactory epithelia feature two more OSN types: crypt cells, possessing microvilli and cilia and expressing apparently only a single olfactory receptor, the V1R-related ORA4 (Ahuja et al., 2013) and the recently described kappe neurons (Ahuja et al., 2014), which only exhibit microvilli, but lie in similar superficial position like crypt cells. Presently, the type of OSNs detecting kin specific odor are unknown. In order to investigate this, we differentially activate OSNs and show neuronal activity reflected by an increase in pERK (phosphorylated extracellular signal regulated kinase) after odor stimulation. To this aim, we stimulated imprinted larvae with food and conspecific odor at different exposure times and identified activated OSNs. We could show that quantity of activated cells is independent of stimuli duration but show different OSN type activation after different stimuli. Furthermore, exposure to kin odor leads to strong response of crypt cells in imprinted larvae, whereas non-imprinted larvae do not show crypt cell activation. This indicates a possible role of crypt cells in olfactory imprinting and subsequent kin recognition.

P10 - The vomeronasal system mediates sick conspecific avoidance.

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While sociability offers many advantages, a major drawback is the increased risk of exposure to contagious pathogens, like parasites, viruses or bacteria. Social species have evolved various behavioral strategies reducing the probability of pathogen exposure. In rodents, sick conspecific avoidance can be induced by olfactory cues emitted by parasitized or infected conspecifics. The neural circuits involved in this behavior remain largely unknown. We observed that olfactory cues present in bodily products of mice in an acute inflammatory state or infected with a viral pathogen are aversive to conspecifics. We found that these chemical signals trigger neural activity in the vomeronasal system, an olfactory subsystem controlling various innate behaviors. Supporting the functional relevance of these observations, we show that preference towards healthy individuals is abolished in mice with impaired vomeronasal function. These findings reveal a novel function played by the vomeronasal system.

P11 - From mouse to man: bacterial signal peptides provide a new mechanism for sensing pathogens.

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There is growing evidence that the sense of smell contributes to the defense against pathogens. However, the underlying physiological and molecular mechanisms remain unclear. Our goal is to identify the molecular machinery involved in odor-mediated defense against pathogens by combining high-throughput receptor pharmacology, cellular physiology and behavioral tests. Here, we propose that recognition of bacterial signal peptides by mouse and human formyl peptide receptors (FPRs) represents a novel mechanism for the detection of pathogens by both the immune- and olfactory

systems. The recent identification of members of the FPR G-protein-coupled receptor family in the vomeronasal organ (VNO), a key organ for odor-guided behaviors, offers an exciting opportunity to study the molecular basis of olfactory pathogen sensing. The well-documented contribution of FPRs for human immune defense suggests that vomeronasal FPRs in mice may also be used for olfactory pathogen sensing and/or avoidance of sick conspecifics. During the past years, we established high-throughput assays that permit the functional in vitro analysis of FPR receptors and are suitable to examine primary cells such as sensory neurons and leucocytes. Systematic structure-function analyses of 20 FPRs from humans, mice and four other species with a large agonist panel showed that the pharmacological profile of VNO-FPRs differs considerably from that of structurally related FPRs in the immune system (Bufe et al., JBC 2012). During the course of these studies, we identified a set of agonist properties, common to both VNO and immune FPRs that show typical features of a pathogen-associated molecular pattern (Bufe et al., JBC 2015). Guided by these results, we discovered that bacterial signal peptides, normally used to translocate proteins across cytoplasmic membranes, are a vast natural family for FPR activators that trigger human and mouse innate immune responses. With at least 175,542 predicted sequences, bacterial signal peptides represent one of most complex classes of G-protein-coupled receptor agonists currently known. Intriguingly, bacterial signal peptides are capable of activating both FPRs from the immune system and the VNO. Although some human and mouse immune FPRs seem to be broadly activated by signal peptides, the mouse vomeronasal receptor mFpr-rs1 and its human sequence orthologue hFPR3 are far more selective. Possibly, FPR1 and FPR2 function as more general sensors of bacteria, whereas hFPR3 and mFpr-rs1 focus on the detection of specific sets of bacterial pathogens. To demonstrate conclusively that bacterial signal peptides are involved in odor-mediated defense against pathogens, cellular and behavioral analyses in mice using these peptides will be required.

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P12 - The G-protein, g-subunit, Gγ8, is expressed in the olfactory and habenular system in mice.

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The habenular system involved in the pathophysiology of psychiatric disorders as schizophrenia and depression and in the impulsive/compulsive behaviors induced by drug addiction. The role of habenula in olfaction is debating. In zebrafish, where the habenular structures are asymmetric, receiving inputs from visual (left habenula) and olfactory (right habenula) stimuli, neurons of the right habenula respond to alarm pheromones eliciting fear behaviors. In rat, lesions of the olfactory bulb elicit habenular degeneration and the onset of neuropsychiatric like disorders. Moreover, predator odors, as cat fur, induce cFos expression in the medial habenula of rat. Recently, we have characterized the physiological role of the olfactory G-protein g-subunit, Gγ8, in the mouse. Gγ8 knockout mice show impaired pheromonal transduction mechanisms leading to alteration of sex specific behaviors. Since an orthologue of Gγ8 is reportedly expressed in the habenula of zebrafish, we have systematically investigated Gγ8 expression in the central nervous system of the mouse. Our results reveal that, in the brain, Gγ8 is exclusively expressed in the medial habenula as well as in the fasciculus retroflexus that connects the medial habenular neurons to the interpeduncular nuclei. Thus, by means of the Gγ8 knockout model, we examine the role of this protein in the habenula mediated behaviors and explore relationship between the habenular and olfactory systems.

P13 - Function study of ionotropic receptor in host odor detection in Anopheles sinensis.

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Mosquitoes are generally considered the most harmful vectors of many disease caused by viruses and parasites due to their blood-feeding behavior. Indeed, they highly depend on odor cues for host preference. However, some important human kairomones such as lactic acid and ammonia which impact on the host-seeking behavior of mosquitoes can not activate any odorant receptors (ORs) and gustatory receptors (GRs). Ionotropic Receptor (IRs), a subfamily of ionotropic glutamate receptors (iGluRs) have been identified as a new family of chemosensory receptors in most insects. Up to two or three IR genes were identified in each ORN which do not express members of OR and GR gene families, and two broadly expressed IRs are hypothesized the function as coreceptors in the OR repertoires. Therefore, IRs are assumed to be responsible for some important host odor detection. We have identified 35 predicted genes in the genome, and screened 7 IR genes expressed in the antenna of Anopheles sinensis, an Asiatic mosquito species which transmits some of the most prevalent human parasitic diseases. Furthermore, RNA in situ hybridization, single-sensillum recordings(SSR) and co-expresses these IR genes with a companion receptor in living cells are being constructed, in order to identify their distribution and host preference function. This study further our understanding of the molecular basis of olfactory-driven behaviors in mosquitoes and lay the foundation to control and prevent the transmit of infectious diseases.

P14 - Anterior piriform cortex activity induced by in vivo optical stimulation of the mouse olfactory bulb.

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In order to improve strategies to study information transfer from the olfactory bulb to the anterior piriform cortex (aPCx), we set up an optogenetic method combining optical stimulation of the bulb with in vivo juxtacellular recordings from single neurons in the aPCx of mice under urethane anesthesia. Patterns of blue light were projected onto the bulb surface using a custom-built system based on a digital mirror device. We used two mouse strains: OMP-ChR2-YFP and Thy1-ChR2-YFP mice expressing ChR2, respectively, in all mature olfactory sensory neurons (OSNs) (Smear et al., 2011) or only in mitral and tufted cells (M/Tc) in the bulb under control of the Thy1 promoter (Arenkiel et al., 2007). Optical stimulation of the olfactory bulb allowed us to drive neurons in deep layer two and layer three of the aPCx in both strains, but we found different patterns of responses. Optical stimulation of the OSNs-terminals in the bulb requires, in our condition, at least 60 ms length of stimulus to drive individual neurons in aPCx. The evoked action potentials (APs) have, in average, 50ms latency from stimulus offset, and the AP rate correlates with the length of the stimulus. In Thy1-ChR2-YFP mice, 20 ms full-field stimulus was already enough to raise reliable APs with, on average, 12 ms latency from stimulus onset. The stimulation efficacy and latency was insensitive to the length of the stimulus but dependent on stimulus size (a bulb surface area >200 μm2). Sparse stimuli of variable size or dense white noise stimuli were used to find spots activating or inhibiting individual aPCx neurons in Thy1-ChR2-YFP mice. Despite a significant increase of firing rate of aPCx neurons during dense noise stimulation, spike triggered averaging analysis rarely revealed specific patterns of bulb activity. These results are consistent with the notion that several glomerular modules have to be coactive to effectively drive cortical neurons and that the stimulation efficacy of individual patterns varies substantially. Our data show application of complementary tools to investigate olfactory physiology at the upper stage of the brain, dissecting the main olfactory pathway with optogenetics and electrophysiology.

P15 - Muscarinic receptors 2 and 5 mediate a negative autocrine feedback mechanism in urethral brush cells activated by bitter stimuli.

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Urethral brush cells (UBC) are cholinergic chemosensory cells functionally expressing the canonical bitter and umami taste transduction signaling cascade (α-gustducin, PLCβ2, TRPM5). Recently, we described them as sentinels initiating local or reflexive protective responses at the entrance to the urogenital tract, communicating via acetylcholine (ACh) release. They also respond to ACh by themselves in that the bitter (denatonium) evoked increase in [Ca2+] i is enhanced in presence of a muscarinic/nicotinic blocker cocktail. Consecutively, an autocrine feedback mechanism seems to be involved in the regulation of the system sensitivity. Expression levels of muscarinic receptors (MR) were determined with RT-PCR, qPCR, and Deep Sequencing of murine UBC. Confocal laser scanning microscopy based intracellular [Ca2+]-imaging was used to record the specific response of UBC isolated from MR knockout animals after stimulation with denatonium without and in presence of a muscarinic/nicotinic blocker cocktail. In expression studies, mRNAs coding for all MR were detected, although only those coding for subtypes 2, 3, and 4 were consistently found. Inconsistent detection of M1R and M5R might be due to expression below the detection threshold. Comparable to control animals, M1R, M3R and M4R knockout animals showed an increase in intracellular calcium concentration in response to denatonium. In UBC isolated from M2/3R double knockouts or M5R knockouts, however, the inhibitors did not enhance the bitter response. Our results show an autocrine, cholinergic negative feedback loop on activated UBC mediated via M2R and M5R.

P16 - Effect of oral bolus versus sustained release application of nutrient solution on olfaction.

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During the last decades, adiposity and correlating diseases, like diabetes and hypertension, became a serious problem in our society. Therefore it is very important to obtain more information how nutrients, especially nutrient application influence human satiety, but also our general physiological and sensory response. Olfaction thereby plays an essential role in food enjoyment and intake, thus investigating olfaction, intensity and hedonic rating of odorants in relation to satiety is fundamental to broaden our understanding of the complex interplay between internal sensing and external perception and rating. In our study we analyzed the influence

of bolus versus sustained release application of a nutrient solution not only on satiety but also in relation to olfaction, intensity and hedonic rating of odorants. Therefore ten healthy male subjects were tested on olfactory function by means of identifying their n-butanol threshold, their capability of odor discrimination and odor identification as well as their hedonic rating of odors, both in a fasting as well as in satiated condition. For olfactory testing of the subjects, we applied the commercially available Sniffin' Sticks test (Hummel et al. 1997). Visual analogue scales were used to document satiety, intensity and hedonic rating. Our results demonstrate that application form has a significant influence on hedonic ratings. We also observed significant differences in terms of satiety between both application forms.

Funding acknowledgemet: Part of this study was undertaken within the Neurotrition Project, which is supported by the FAU Emerging Fields Initiative.

P17 - Metamorphotic remodeling of the olfactory organ of the African clawed frog Xenopus laevis.

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The amphibian olfactory system undergoes massive remodeling during metamorphosis. The transition from aquatic olfaction in larvae to semi-aquatic or airborne olfaction in adults requires anatomical, cellular and molecular modifications. These changes are particularly pronounced in Pipidae, whose adults have secondarily adapted to an aquatic lifestyle. In the fully aquatic larvae of Xenopus laevis, the main olfactory epithelium specialized for sensing water-borne odorous substances lines the principal olfactory cavity (PC) while a separate olfactory epithelium lies in the vomeronasal organ (VNO). During metamorphosis, the epithelium of the PC is rearranged into the adult 'air nose', while a new olfactory epithelium, the adult 'water nose', forms in the emerging middle cavity (MC). Here we performed a stage by stage investigation of the anatomical changes of the Xenopus olfactory organ during metamorphosis. We quantified cell death in all olfactory epithelia and found massive cell death in the PC and the VNO suggesting that the majority of larval sensory neurons is replaced during metamorphosis in both sensory epithelia. The moderate cell death in the MC shows that during the formation of this epithelium some cells are sorted out. Our results show that during MC formation some supporting cells, but not sensory neurons, are relocated from the PC to the MC and that they are eventually eliminated during metamorphosis. Together our findings illustrate the structural and cellular changes of the Xenopus olfactory organ during metamorphosis.

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P18 - Adjustment to Olfactory Loss in Patients with Parkinson's Disease.

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Though it is well known to the scientific community that patients with Parkinson's Disease (PD) suffer from decreased olfactory ability, an individual patient may well be unaware of their own loss (Anchuri et al 2015; Doty et al 1988). Despite a high level of anosognosia for their olfactory loss, it is possible that people with PD still feel the effects of dysosmia in terms of quality of life decrements. Further, it is possible that these patients cope with olfactory loss by developing depression, anxiety, and/or devaluing olfactory stimuli in their daily lives. The aim of the present study was firstly to determine whether there was a relationship between olfactory ability, quality of life, and the importance of olfactory stimuli, and secondly to ask whether people with PD differed from controls on those factors. Nineteen non-demented PD patients and 19 age-matched control participants were each given the UPSIT (Doty et al., 1984), the Importance of Olfaction survey (IO; Croy et al., 2010), and completed questionnaires on quality of life (the PDQ-8, Jenkinson et al., 1997), depression (BDI-II) and anxiety (BAI). Across participants, the lower olfactory function was significantly correlated with the importance of olfaction in everyday life ($r = .50$, $p < .01$) and the quality of life ($r = -.58$, $p < .01$). The importance of olfaction was reduced in people with PD compared with controls [$t(36) = -3.12$, $p < .01$], as were the UPSIT scores [$t(36) = -9.03$, $p < .01$] and the quality of life [$t(36) = 6.22$, $p < .01$], while anxiety [$t(36) = 2.82$, $p < .01$] and depression [$t(36) = 1.79$, $p < .082$] scores were elevated. Results support literature that indicates that decreased olfactory ability is associated with decreased quality of life (Kurtz et al., 1995) and decreased importance of olfactory stimuli (Croy et al., 2011). Even though patients with PD cope with their olfactory loss by devaluing the importance of odorants in their environment, their elevated depression and anxiety symptoms might be at least partly associated with their dysosmia.

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P19 - Co-expression of Anoctamins in cilia of olfactory sensory neurons.

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Vertebrates can sense and identify a vast array of chemical cues. The molecular machinery involved in chemodetection and transduction is expressed within the cilia of olfactory sensory neurons. Currently, there is only limited information available on the distribution and density of individual signaling components within the ciliary compartment. Using super-resolution microscopy, we show here that cyclic-nucleotide-gated channels and calcium-activated chloride channels of the anoctamin family are localized to discrete microdomains in the ciliary membrane. In addition to ANO2, a second anoctamin, ANO6, also localizes to ciliary microdomains. This observation, together with the fact that ANO6 and ANO2 co-localize indicates a role for ANO6 in olfactory signaling. We show that both ANO2 and ANO6 can form heteromultimers and that this heteromerization alters the channels' physiological properties. Thus, we provide evidence for interaction of ANO2 and ANO6 in olfactory cilia, with possible physiological relevance for olfactory signaling.

Funding acknowledgement: This work was supported by the Deutsche Forschungsgemeinschaft SPP 1392: "Integrative Analysis of Olfaction"

P20 - Probing emotional and behavioral effects of oral exposure to bitter and sweet tastants.

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Bitter taste is one of the basic taste modalities, and is generally thought to guard against consuming harmful substances. The role of bitter taste as a warning signal implies that oral exposure to bitter taste might elicit aversive behavior. Expressions of such behavior may include risk aversion, reduced trust, reduced attraction to other people, and negative emotions. The hypotheses regarding these behavioral effects were tested using 190 participants who were asked to "sip and spit" either water or bitter, bitter-sweet, or sweet solutions, and to perform several tasks. No significant differences in tasks related to risk aversion, trust and attraction were found between the bitter solution mouth rinse group and the rest of the participants. However, we found that bitter taste mouth rinse elicited negative feelings and reduced positive ones, compared to control. Interestingly, the taste effect on emotions was asymmetric: oral exposure to sucrose did not elicit significant effects on emotions compared to control. Nevertheless, sucrose negated the effects of the bitter substance when the two were mixed in a single solution.

These results are particularly interesting given the unpaired observations study design and the fact that the subjects were unaware of questions under investigation.

P21 - Odorant-odorant metabolic interactions could facilitate the peripheral processing of relevant olfactory signals.

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At birth, newborn rabbits face the critical and vital need to suck. To do so, they are olfactory guided by the mammary pheromone (MP) emitted by all lactating females in their milk. Given the efficacy of the MP to induce nipple searching and oral grasping in neonates, the ad minima hypothesis is that this olfactory signal is efficiently processed from the peripheral level of the olfactory system. This is why, we assess here a more original and innovative hypothesis according to, the peripheral level would host olfactory sensory neurons (OSNs) expressing MP sensitive receptors, but also supplemental mechanisms which could optimize its detection. For instance, in the olfactory mucosa perireceptor environment, odorant metabolizing enzymes (OMEs) ensure odorant deactivation and clearance. OMEs are identical to enzymes involved in systemic detoxification (e.g., drug-drug interactions). OMEs could interact with the olfactory perception since first, they de facto modify the bioavailability of odorant molecules surrounding OSNs and second, most of natural olfactory stimuli consist in complex blends of different volatile chemicals. Thus, OME could lead to odorant-odorant interaction and result in a differential increase of one molecule bioavailability over another molecule. Therefore, in conjunction with neural processing, OMEs could participate to both quantitative and qualitative modulations of the olfactory perception. We previously showed that the OME metabolism of the MP involves glutathione transferases. Here, using HPLC analysis on olfactory mucosa homogenates, we show that competitive metabolic interactions with other aldehydes partly abolishes glutathione conjugation activity toward the MP, and confirm this mechanism with an original ex-vivo method, headspace GC analysis on olfactory mucosa fresh explant. The MP potency to trigger the sucking behaviour in rabbit newborns gave us access to the impact of odorant-odorant metabolism on its perception: when the MP is mixed with 2M2P (OME competitor aldehyde), neonates display sucking behaviour for this mixture; strikingly, in mixture, the MP triggers sucking behaviour for lower concentrations than it does alone, i.e. the MP perception threshold seems to be improved. Furthermore, electro-olfactograms recorded ex-vivo in neonates show, first, that the olfactory mucosa is especially sensitive to aldehydes and second, that binary mixtures of MP+2M2P evoke larger responses than the MP

alone. Such an additive effect supports a potent increase in MP bioavailability to the detriment of 2M2P, paired or not with an especially high affinity of olfactory receptors for the pheromone. These multi-disciplinary results constitute the first demonstration of odorant-odorant metabolic competition mechanisms in situ. OMEs appear as good candidates to interfere with peripheral odorant processing since they can change the bioavailability of odorants and thus, modify their detection, perception and resulting behaviours.

P22 - Taste and palatability of Pyrophosphates by rats: a sensorial qualitative and quantitative approach.

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SPF - Diana Pet Food, Elven, France; Opertech Bio Inc., Philadelphia, USA

Sodium pyrophosphates are well known ingredients used ubiquitously as cat food palatability agents. In 2010, Brand and Bryant (WO 2012/013480) demonstrated that at least the feline umami receptor was involved in their perception. Furthermore, preference tests and electrophysiology experiments on rats established pyrophosphates were highly palatable to them and suggested that this preference was not only due to their salty taste (McCaughey et al., 2007). Unfortunately, there is no method to evaluate cats' taste quality perception, but Palmer et al. reported in 2013 the development of a novel sensory assay capable of simultaneous measurement of taste quality and palatability with high throughput capacity, using rats in operant taste discrimination paradigm. We sought to further investigate taste and palatability of pyrophosphates using this technology to describe pyrophosphates taste quality. Five cohorts of four rats were trained to each discriminate one of the five basic tastes. Once ready, the rats were given series of sodium pyrophosphates solutions at various concentrations (2, 10 and 30mM) so as to identify their global taste profile. Then, the salty cohort was used to evaluate if pyrophosphates preference was mainly brought by their saltiness by evaluating the same solutions after inhibiting rats salty taste receptors with 100µM of amiloride. This study let us validate that sodium pyrophosphates are mainly perceived by rats as salty and significantly preferred to water. However, once the salty taste is inhibited, it is still preferred to water. We can therefore conclude that pyrophosphates have a distinctive taste besides the salty one, and that they might act on a separate gustatory transduction mechanism than those associated with the five prototypical taste qualities.

P23 - Gene expression tph-1 in mouse cell enterochromaffin, by administering dietary fiber from blackberry. Ziracuaretiro produced in Michoacán.

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Serotonin plays multiple functions in organisms including regulation of intestinal functions, serotonin is synthesized by the enzyme tryptophan hydroxylase (TPH), which has two isoforms TPH-1 and TPH-2, the TPH-1 is expressed in cells small bowel enterochromaffin mammals, the TPH-1 is encoded by the tph-1 gene. Dietary fiber promotes bowel function, but so far there is no evidence of the involvement of dietary fiber and induction of gene messenger RNA enzyme TPH-1, so that the work aims to establish the expression ratio tph-1 gene encoding the receptor and a butyrate concentration of dietary fiber Bramble from fruit, as a fiber source. Evidence that dietary fiber from R. fruticose at a concentration of 10% is an alternative for the induction of mRNA of the gene tph intestinal-1 level in mouse C57BL / J6 strain shown mammals. Therefore puts the dietary fiber of this fruit as a new alternative as a food derived from the fruits that are not sold in fresh for export. It was determined that after 14 days of administration of dietary fiber R. fruticosus at a concentration of 10% the maximum expression of mRNA gene tph-1 in mouse colon intestine of the C57BL / 6 strain is achieved.

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P24 - Slow Rhythmic Burst Activity in the Rodent Accessory Olfactory Bulb.

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Within the mouse vomeronasal system, the accessory olfactory bulb (AOB) is the first site of central information processing. Mitral cells (MCs), the AOB's projection neurons, receive sensory input from vomeronasal neurons and relay processed signals to higher brain areas. A subpopulation of MCs exhibits intrinsic oscillatory activity that is insensitive to blockade of fast synaptic transmission and manifests in periodical bursting. A second MC subpopulation displays entrained oscillatory activity that depends on an intact glutamatergic network. In this study, we use a multi cell fluorescence imaging approach to monitor oscillatory activity in acute mouse AOB tissue slices. Bulk loading of synthetic calcium indicator dyes allows parallel observation of various MC activity patterns. Pharmacological inhibition of fast GABAergic and / or glutamatergic transmission supports the notion of an excitatory feed-forward AOB network that

synchronizes AOB projection neurons. Thus, our data provides a mechanistic basis for slow rhythmic activity in AOB information processing.

P25 - Anatomical and functional organization of the Drosophila antennal lobe: glomerular convergence and divergence.

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Chemical environments consist of a vast, complex and fast changing array of volatile cues with differing ecological relevance. To orient within this plethora of volatile cues, the vinegar fly Drosophila melanogaster possesses an elaborate olfactory system consisting, on the periphery, of a set of four sensilla classes present in stereotypical patterns on the 3rd antennal segment and the maxillary palp. Each sensilla type, either antennal basiconic, trichoid, coeloconic or palp basiconic, houses one to four olfactory sensory neurons (OSN), each expressing a known repertoire of one or two olfactory receptors with a unique molecular receptive range. OSNs expressing the same receptor converge in the protocerebrum forming 54 discrete spherical structures, the glomeruli as units of the antennal lobe (AL), where they synapse with omniglomerular local interneurons and uni- or multiglomerular projection neurons. In our study we investigate the concise basis of this neuronal network. Most glomeruli, except the putative pheromone-receptive structures, are often thought to be part of a very homogeneous cluster of AL subunits solely separated by the receptor repertoire of innervating OSNs. Although an increasing group of non-pheromonal glomeruli serving very specific tasks has been recently discovered, their potentially differing anatomy is usually neglected. Here we display the heterogeneous nature of the glomeruli regarding their specific neuronal input and output quantity as well as their in vivo volume. Based on the morphological data we show that described functional differences can also be correlated to anatomical specifications.

P26 - Testing the vibration theory of olfaction in the honeybee brain.

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The standard theory of primary olfaction assumes that odour receptors sense molecular shape and binding properties of odourants via a key-lock mechanism. Discrepancies regarding consistent shape-odour relations motivated an alternative proposal of a vibration theory of olfaction, where a spectroscopic mechanism probes the molecular vibrations of the odourants. Here we present first in vivo functional imaging data indicating a contribution of molecular vibrations to odour coding in the honeybee brain. Isotopomer pairs, chemically identical but with differing vibrational spectra, elicited different odour response maps in the antennal lobe. The isotope sensitivity is highly consistent across individuals and not limited to single receptor types. Furthermore, a clear relation between the diversity of odour codes and vibrational spectra was observed.

P27 - Differential modulation of human taste receptors by polyphenolic bitter blocking compounds.

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Perception of bitter taste has been recognized as an essential early warning system against the intake of potentially harmful substances. However, in some instances, bitter compounds have beneficial effects on human physiology and may therefore be advantageous ingredients of human food or drugs. In these cases, bitter masking compounds are utilized to enhance palatability or – in case of drugs – to increase patients' compliance. In past studies, we described several polyphenolics such as homoeriodictyol, eriodictyol and structural relatives as potent bitter taste reducing compounds in sensory and structure activity approaches. In order to elucidate the molecular mechanism by which these compounds act, we investigated their effects on the matrix of human bitter taste receptors (TAS2Rs), each individually expressed in HEK239 cells. The bitter taste reducing compounds were each tested on the range of 21 TAS2Rs alone as well as in combination with known activators of the respective receptors. Whereas all substances investigated here inhibited the activation of a specific set of bitter receptors, they were all found to activate another subset of the TAS2Rs. The pattern of activation/inhibition was found to be highly substance-specific, with a certain degree of correlation of chemical similarity to the detected activity pattern. The results of this study reveal a first insight into the complexity of bitter receptor activation by distinct compounds and the – yet hypothetical – in vivo interplay of the different members of the TAS2R family in perceiving bitter taste. The study paves the way for further research into the molecular processes and cross-talks that lead from the detection of a molecular signal on the apical surface of a taste cell to the generation of a "bitter signal" that is transmitted to the brain.

P28 - Contribution of TMEM16A to the Calcium-Activated Chloride Current in Mouse Vomeronasal Sensory Neurons.

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The vomeronasal organ is involved in the detection of pheromones. The binding of pheromones to receptors in microvilli of vomeronasal sensory neurons (VSNs) activates a phospholipase C signaling cascade producing the entry of Na+ and Ca2+ through TRPC2 channels. In this study, we have used patch-clamp recordings to provide a characterization of currents activated by Ca2+ in isolated mouse VSNs. In whole-cell recordings, we found that 70% of VSNs have a Ca2+-activated current Cl^- current. Inside-out patches from dendritic knobs confirmed the presence of Ca2+-activated Cl^- channels in the knobs and/or microvilli of mouse VSNs. Comparing our results with the properties of heterologous expressed TMEM16A and TMEM16B Ca2+-activated chloride channels, which are co-expressed in mature VSNs, we found a closer similarity to those of TMEM16A. Taking advantage of the expression of the olfactory marker protein (OMP) in mature VSNs, we selectively knocked out TMEM16A in these neurons, by crossing floxed TMEM16Afl/fl mice with mice expressing Cre recombinase under the control of the OMP promoter. The specific ablation of TMEM16A in mature VSNs was confirmed by immunohistochemistry; while the expression of other membrane proteins present in the microvilli, such as TRPC2 and TMEM16B, remained unaltered. Whole-cell recordings from TMEM16A conditional knockout mice showed that Ca2+ activated currents were abolished, while voltage-gated inward currents were present in VSNs. These results demonstrate that the TMEM16A protein is expressed in mature VSNs and is an essential component of Ca2+-activated Cl^- currents in mouse VSNs.

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P29 - Plant odor responses of antennal lobe projection neurons measured via calcium imaging.

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The olfactory system possesses evolutionary well conserved arrangements, showing striking similarities across living organisms from insects to mammals. Due to its exquisite sense of smell and relatively accessible neural system, moths are suitable models for exploring basic principles underlying olfactory information processing. As in other insects, antennal sensory neurons of the moth convey odor information directly to the antennal lobe, the primary olfactory center of the brain. Here, the sensory terminals make synapses with 2nd order neurons in characteristic spherical structures termed glomeruli. The male-specific neurons, which are housed in long antennal hairs, target a small number of enlarged glomeruli situated dorsally in the antennal lobe, whereas plant odor neurons, being present in both sexes and encapsulated in short hairs, project to the numerous ordinary glomeruli. After being processed in the antennal lobe, the odor information is transferred via parallel antennal-lobe tracts (ALTs) to two higher integration areas, the calyces of the mushroom bodies and the lateral horn. In moths, the connection between the antennal lobe and the calyces is maintained primarily by one of these paths, the medial ALT. In the study presented here, we have used calcium imaging to measure activation patterns of antennal-lobe glomeruli in the heliothine moth. A great fortune of studying this subfamily of noctuid moths is that several odor ligands, including pheromones and plant odors, have been identified in these species. By applying a calcium-sensitive dye into the junction between the medial and lateral calyx, we were able to measure odor-evoked responses from the uni glomerular projection neurons confined to the medial ALT specifically. The stimuli which included four plant odorants are previously shown, via single cell recordings combined with gas chromatography, to act as ligands on distinct populations of sensory neurons (linalool, geraniol, farnesene, carene; Røstelien et al. 2005, Chem. Sens.). Antennal stimulation with the odors, which were applied as single substances and mixtures, offered the opportunity to measure spatio-temporal activity patterns of the glomeruli. In general, odor-specific response patterns comprised both excitatory and inhibitory glomerular activity. In line with calcium imaging studies of other insect species, we observed mixture interactions, particularly in the form of suppression upon stimulation with certain binary mixtures. The particularity of these mixture-interactions indicates irregular inhibitory interglomerular connections, which is likely to reflect the evolved ecological interplay between host-plants emitting the respective odors and the heliothine moth.

P30 - Loss of brush cells and taste cells in the gastrointestinal tract alters energy metabolism in Skn-1-deficient mice.

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The gastrointestinal tract senses nutrients to regulate food intake and energy metabolism. To reveal the underlying molecular mechanisms, we investigated phenotypes of Skn-1 knockout (KO) mice, in which specific types of taste cells are

eliminated. Skn-1 KO mice gain reduced body weight with lower body fat percentage due to higher energy expenditure, in spite of unaltered food intake. Serum levels of total ketone bodies and mitochondrial DNA content in skeletal muscle are higher in Skn-1 KO mice. When fed a high fat diet, catecholamine secretion is augmented, while insulin resistance and impaired glucose tolerance are ameliorated in Skn-1 KO mice. Skn-1 KO mice show reduced rise in plasma insulin levels after gastric glucose gavage. Finally, we found that brush cells are abolished in the small intestine of Skn-1 KO mice. These results suggest the existence of novel pathways originating from brush cells and taste cells to maintain energy homeostasis.

P31 - The Neural networks underlying the liking and wanting responses to food odors are modified in anorexia nervosa.

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Anorexia nervosa (AN) is an eating disorder which has the highest mortality rate of any mental illness. The understanding of the neural substrates and processes underlying this disorder constitutes an important step towards their treatment. Research suggests that the brain reward responses to food stimuli might be a critical factor controlling both quantitative and qualitative food intake. We addressed the issue of whether AN is related to changes in brain reward processing. Event-related fMRI was used to compare responses to food odors (stimuli for both anticipatory and consummatory food reward processes) in 12 healthy women (Ctr) and 14 women with AN. Each participant was scanned during two sessions over two consecutive days, one day in the pre-prandial state and the next day in the post-prandial state. During each session, BOLD responses to liking and wanting judgments (representing the two components of the reward process) were acquired in two separate runs. Liking and wanting judgments were rated for each food odor with a 5 key-press button box. Functional analyses showed that 1) in all experimental situations (liking and wanting tasks, hunger and satiate states), AN women had significantly lower BOLD activity in the precuneus area, which is a cortical region involved in self-perception; 2) AN women had higher activity in regions involved: a) in visual perception, such as the occipital cortex (especially at hunger state); in body image, such as fusiform gyrus (especially for the wanting task); in high control processes, such as the polar medial prefrontal cortex; 3) the Ctr group evinced higher activity in the parieto-frontal inhibitory control regions, such as the supra-marginal and angular cortical regions and the dorsolateral prefrontal cortex. Our findings suggest that AN is associated with changes in the brain processing of odorant stimuli, and that food stimuli may activate brain networks involved in self-perception and executive functions.

P32 - Odor evoked disgust and the immune system.

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Human beings respond with the feeling of disgust when confronted with potential pathogen transmitters such as feces, decayed food, and sick or wounded individuals. Disgust is assumed to guide pathogen avoidance behaviors, to impact health anxiety, and it has been shown to trigger immune responses. We investigate the relationship between disgust related predispositions, the level of perceived disgust evoked by olfactory stimuli, and the reactivity of the immune system, as measured by cytokine levels in blood and saliva, in 19 healthy individuals using a within-group design. Participants were exposed to neutral and disgusting odors at two separate occasions respectively. Olfactory-evoked disgust was measured on visual analogue scales and ratings were compared with cytokine levels in blood (TNFa, IL-6) and saliva (TNFa, sIgA), as well as with individual predispositions to perceive disgust (DS-R, disgust scale revised), and health anxiety (HAI, health anxiety inventory). DS-R and HAI measures were significantly related to ratings of neutral but not disgusting odors. Furthermore, the relationship between these outcomes and the activation of the immune system will be analyzed and results will be presented at the conference.

P33 - Topographic representation of space in mouse olfaction.

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The ability to localize stimuli is crucial for survival, and both the visual and auditory systems have been exquisitely refined to preserve the spatial information in incoming sensory data. However, it is not known whether such capabilities exist for the olfactory system. Here, we report that head-fixed mice discriminate odor direction in both horizontal and vertical planes. Surprisingly, mice with a single unobstructed nostril could reliably distinguish odor plumes from different positions along the vertical axis. To explain this observation, we tested whether near-nostril space is topographically mapped to distinct regions of the main olfactory epithelium (MOE). By constructing transparent three-dimensional physical models of the mouse nasal cavity to visualize flow, we found

that in addition to separate sampling by the two nostrils, inhalation through each nostril preserved separate dorsal and ventral odorant streams, mapping to lateral and medial MOEs, respectively. Electroolfactogram (EOG) recording showed that the lateral and medial MOEs responded differentially to odor from different elevations, with the size of the difference dependent upon sniff rate. These results demonstrate that mice use both bilateral sampling and lateral-medial MOE contrast to localize odor, and resolve many long-standing mysteries about the anatomical organization of the olfactory periphery and of sniffing behavior.

P34 - Function of contra-lateral interactions between olfactory bulbs.

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Similar to vision or audition, the peripheral olfactory system consists of a pair of sensory organs. Olfactory information coming from a given nostril is sent to the ipsilateral olfactory bulb and then transmitted to the olfactory cortices. This suggests an independent and somehow redundant encoding of olfactory information by two parallel olfactory channels. Previous anatomical studies suggest that two parallel olfactory channels can interact at least through contralateral top-down projections from the olfactory cortex. However the extent of these contralateral interactions between parallel olfactory channels and their function in odor processing is not studied in detail. In order to identify the anatomical basis of connections between two olfactory bulbs we first used dye electroporation. Our results showed that the two olfactory bulbs are strongly connected in addition to the contralateral projections from the cortical areas. We observed that while top-down projections from the olfactory cortices are rather diffuse and mostly terminating at the inhibitory granule cells layer, the direct connections between olfactory bulbs are layer specific and mostly terminating at the mitral-cell/glomerular layer of the olfactory bulb. Using targeted dye electroporation in genetically identified glomeruli, we showed that these direct connections between olfactory bulbs are topographically organized, where sister glomeruli in contralateral olfactory bulbs are strongly linked with direct mitral cell projections. In order to study the function of these direct and indirect interactions between two parallel olfactory pathways, we used a combination of intracellular recordings, electrical micro-simulation and two-photon calcium imaging. Our results showed that information coming from one olfactory bulb can significantly alter odor responses in the contralateral olfactory bulb, eliciting a balance of excitation and inhibition. Interestingly, low intensity contralateral stimulations elicit mostly excitation whereas high intensity contralateral stimulations also elicit inhibition, suggesting a role for contralateral connections in regulating gain control.

P35 - Age-related change in the time course of perceived intensity of continuously presented odors.

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Olfactory function generally decreases with age. However, the ability of elderly people to sense a continuously presented odor, such as a gas leak, remains poorly characterized. In this study, we investigated whether the time course of perceived intensity of a continuously presented odor differs among young, middle-aged, and older adults. Three odorants were used: ethyl isobutyrate (EI), tertiary-butylmercaptan (TBM), and cyclohexene (CH). Participants evaluated the perceived intensity of a presented odor for 600 seconds. Odors were presented at a constant concentration for 480 seconds; pure air also flowed for 20 seconds before, and 100 seconds after odor presentation. For time–intensity evaluation, we used the sliding lever. This sliding lever had a movable range of 30 cm in the horizontal direction. The position of the sliding lever, corresponding to the evaluation value, was converted into voltage; voltages were acquired every 50 ms as digital data using a computer equipped with an analog-to-digital converter interface. During time–intensity evaluation, participants were seated in a chair with the face oriented approximately horizontally and the nose and mouth covered by the mask. We set up a liquid crystal display about 150 cm in front of the participant, which provided visual feedback of the evaluated intensity based on the 6-point LMS. Furthermore, because we did not inform participants about duration of evaluation, we displayed the elapsed and remaining evaluation time on the screen. Participants did not know the timing of the switches between odor and pure air, or the duration of odor presentation. Remarkable differences in the time course of perceived intensity among groups were observed during the air presentation (no odorant) period following odor presentation. After ~60 seconds elapsed after the end of odor presentation, older adults perceived an increase in the intensity of EI and TBM. Because this phenomenon was not observed in the time course of perceived intensity for young and middle-age adults, we concluded that this phenomenon might represent a sensory property or olfactory recognition, peculiar to elderly people in the context of a continuously presented odor.

P36 - Sensitization to L-Felinine in Mice is Correlated with Elevated Fos-Immunoreactivity in Accessory Olfactory Bulb.

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Felinine is a unique sulfur-containing amino acid found in the urine of domestic cats. Sulfur-containing volatile felinine

derivatives may represent pheromones used as territorial markers for conspecific recognition or reproductive purposes by mature cats (Miyazaki et al. 2008). Our data confirm that these species-specific compounds may be used also by mice to recognize potential predators and their physiological status; they may affect reproductive output in mice (Voznessenskaya 2014). Neonatal exposures to odorants influence the function of the olfactory system to produce changes in responses to these stimuli later in life. Modulation of sensitivity by environmental factors to some odorants considerably influences an organism's adaptability. Our earlier studies showed that regardless of the odorant, early exposure to it resulted in an increase in a rodent's sensitivity to that stimulus (Voznessenskaya et al. 1995). Exposure to the odorant during two weeks after the pups opened their eyes appeared to induce the greatest level of sensitization, suggesting a sensitive period to such stimuli (Voznessenskaya et al. 1999). The specific aim of our current study was to examine whether early olfactory experience of mice with chemosignals of cats during "critical" period for sensitization to odors may affect sensitivity to target odors later in adulthood and whether these changes in sensitivity correlated with neural activation in olfactory bulbs. Olfactory thresholds to cat urine/ L-felinine were measured using an eight-channeled olfactometer (Knosys, USA). Exposures of mice to cat chemical cues (urine or L-felinine) during "critical period" significantly lowered the olfactory thresholds to cat urine ($n=10$, $p<0.05$) as well as to L-felinine ($n=10$, $p< 0.01$) relative to controls exposed to tap water during the same period. Elevated sensitivity to cat's chemosignals is adaptive for detection of the predator under natural conditions. We performed immunohistochemical studies to identify neural substrate involved in reception and analysis of L-felinine. Mice were exposed intermittently (50% duty cycle each minute) to 0.05% L-felinine ($n=8$) or clean air ($n= 8$) for 45 minutes prior to perfusion for immunostaining of the olfactory bulbs for Fos. Bulbs were sectioned at 30μ and stained (c-Fos (4) sc-52, Santa Cruz Biotechnology, USA; Alexa Fluor ®594, Life technologies, USA). Sections were analyzed using All-in-One Fluorescence Microscope Keyence Bz -9000 (Keyence, Japan) with software. Specific pattern of activation was recorded in accessory olfactory bulb (AOB). Neonatal exposures to L-felinine caused significant increase in number of Fos-positive cells in AOB in response to stimulation with L-felinine ($n=8$, $p < 0.01$). Also we recorded an increase of activated area ($n=8$, $p< 0.001$). Sensitization to L-felinine in mice correlated with elevated Fos-immunoreactivity in AOB in response to stimulation with the compound.

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P37 - L-felinine May Affect Estrous Cycles in the House Mouse.

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Domestic cat (Felis catus) and the house mouse (Mus musculus) may serve as an excellent model for the study of predator-prey relationships including interspecific chemical communication. A long history of coexistence led to the development of mutual adaptations. L-felinine is a unique amino acid found in the urine of domestic cat and select members of the Felidae family. Our previous research showed that L-felinine may play a role of chemical signal in the house mouse. In the current study we examined the influence of L-felinine on regulation of estrous cycles in female mice. We have validated noninvasive estradiol assessment technique in order to monitor estrous cycle phase (ECP). Fecal estradiol levels were determined using ELISA technique (Immunotech, Russia) and standard extraction procedure for each female daily. Vaginal smears were also taken on daily basis to control ECP. We used four groups of mice ($n=38$) at age of three months: (1) continuous action of L-felinine (0.05%; 50 μl) during 12 days; (2) application of L-felinine (0.05%; 50 μl) at regular intervals for duration of two hours on daily basis (total time of exposure during 12 days was 24 hours); (3) spontaneous exposures to L-felinine (0.05%; 50 μl) but the total time of exposure during 12 days was also 24 hours; (4) control (exposure to tap water). We collected fecal samples from each female at the same time of the day for duration of the experiment. Estradiol baseline was calculated individually for each animal; concentrations above the baseline were considered as a beginning of luteal phase of estrous cycle (Brown et.al. 1999, De Bruin et.al. 2014). The data obtained indicate that L-felinine may affect the length of estrous cycle in mice. The number of ovulations in the group of animals under continuous exposure to L-felinine significantly increased (t-test, $p=0.00498$, $n=10$). It appears to be that females habituated to the continuous action of the compound and as a result they exhibited regular estrous cycles. At the same time we observed decline in a number of cycling females in the group 2 (regular application of L-felinine for two hours at the same time of the day) ($\chi 2$, $p=0.0233$, $n=9$). For the group 3 (spontaneous application of L-felinine) we observed only a tendency to decrease in a number of cycling females ($\chi 2$, $p=0.0578$, $n=9$). Different modes of exposure to L-felinine produced different effect on estrous cycles in mice. For further conclusions additional data are required.

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P38 - Brain metabolism correlates with the interest raised by odorant stimulations: a microPET study in rat.

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Odor processing beyond the olfactory bulb (OB) remains poorly documented. This is likely due to the ventral location and the large extent of other olfactory cortical areas. Indeed, traditional methods (2-DG mapping, electrophysiology, optical imaging) are difficult to apply to olfactory structures due to their invasive nature and/or their restrictive field of view. In vivo imaging techniques (fMRI, PET) are currently available in rodents, and overcome above limitations providing repetitive and non-invasive mapping of the whole brain. If fMRI has been successfully used to study odor-elicited activity patterns in the OB, this technic suffers from limitations due to low contrast-to-noise ratio in ventral brain structures and the use of anesthetized animal. In spite of its limited spatial resolution compared to fMRI, µPET allows examination of whole brain function in freely moving animals as the animal is awake during the tracer uptake. The present works reports an initial series of µPET experiments designed to investigate brain areas involved in odor processing during a passive detection task. After [18F] FDG injection, rats were placed in a ventilated Plexiglas cage. Four different odorants were delivered randomly every 3 min and for 10s from the top of the cage for a 50 minutes session. Rat behavioral response to odor was classified into 4 categories from 1 (no reaction) to 4 (rearing and exploring). After FDG uptake, rats were anesthetized with isoflurane to perform a 20 minutes PET scan (4 frames, 159 slices of 128 x 128 voxels with voxel size 0.388 x 0.388 x 0.796 mm). The experiment was repeated two weeks later without odor stimulation to assess a baseline level of activation. Sessions order was reverse for another set of rats. PET data processing was carried out using SPM8. After spatial normalization to a custom PET FDG template, two voxel based statistical analysis were performed i) a two sample paired t-test analysis and contrasting baseline versus odor scan; ii) a correlation analysis between voxel FDG activity and behavioral scores. T-value maps were overlaid onto the MRI template to identify brain structures that showed significant changes. As expected, contrast analysis showed activations in various olfactory cortical areas. Significant increase in glucose metabolism was also found in other sensory cortical areas involved in whisker movements and in several modules of the cerebellum involved in motor and sensory functions. The correlation analysis provided new insight into the results. [18F] FDG uptake was correlated with behavioral response in a large part of the anterior piriform cortex and in some lobules of the cerebellum agreeing with previous data showing that both piriform cortex and cerebellum activity in human could be driven by sniff. The present data demonstrated that µPET imaging offers new perspective for rat behavioral neuroimaging.

P39 - An Increase in Taste Cell cAMP regulates the Postnatal Development of Na+-specific Salt Taste.

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In rats, during a period of 7 to 50 days postnatal, the chorda tympani (CT) taste nerve responses to NaCl increase four folds. This is due to an age-dependent increase in the number of functional epithelial Na+ channels(ENaCs) in the apical membrane of salt sensing TRCs in fungiform (FF) taste buds. To test if a gradual increase in TRC cAMP is involved in the age-dependent maturation of TRC ENaC, we monitored NaCl CT and behavioral responses in 19–23 days old rats before and after topical lingual application of 8-chlorophenylthio(CPT)-cAMP, under open-circuit and lingual voltage-clamp conditions. The ENaC-dependent NaCl CT response was a sigmoidal saturating function of 8-CPT-cAMP concentration (0–20 mM). The data could be fitted to a Hill type equation. The maximum increase in the ENaC-dependent NaCl CT response (230%) was observed between 15 and 20 mM and the half maximum response at 10.9 +/- 1.7 mM 8-CPT-cAMP, with n = 7.2 +/- 3.0, indicating that a high threshold concentration of TRC cAMP must be achieved before an increase in the NaCl CT response is observed. The ENaC-dependent CT response was a linear function of the applied lingual voltage between -60 mV and +60 mV. In 19–23 days old rats the negative of the slope of the normalized ENaC-dependent NaCl CT response as a function of applied voltage, defined as the response conductance, increased by 1350% after lingual application of 20 mM 8-CPT-cAMP. In 2 bottle choice tests, 19–23 day old rats did not discriminate between H2O and 0.15 M NaCl (mean NaCl preference 0.56 +/- 0.027). After 20 mM 8-CPT-cAMP treatment the mean NaCl preference was 0.81 +/- 0.017. In 15 days old rats, gamma-ENaC antibody demonstrated binding to a subset of FF TRCs in the cytoplasmic compartment. Following intraperitoneal administration of arginine vasopressin (1 nM/Kg body weight), a hormone that uses V2 receptor (V2R) and cAMP as a second messenger, induced trafficking of gamma-ENaC to the apical membrane of a subset of FF TRCs. In contrast to the adult rats, no V2R antibody binding in TRCs was observed in 14 days old rats. We conclude that a temporal increase in V2R and cAMP may set the pace for the development of ENaC in young rats.

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P40 - Chorda Tympani (CT) Nerve Responses to Nicotine, Acetylcholine, and Ethanol are Modulated by Nicotinic Acetylcholine Receptor Agonists and Antagonists.

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Nicotine (Nic) elicits bitter taste by activating TRPM5-dependent and TRPM5-independent but neuronal nAChR-dependent pathways. We investigated if nAChRs represent a common pathway through which the bitter taste of Nic, acetylcholine (ACh), and ethanol (ETOH) is transduced. We monitored CT responses in Sprague-Dawley rats, wildtype (WT) and TRPM5 knockout (KO) mice following lingual stimulation with Nic, ACh and ETOH in the absence and presence of nAChR modulators. Stimulating the tongue with Nic (0–20 mM) elicted dose-dependent increase in the CT response in WT and KO mice with 40% lower response in KO mice. Mecamylamine (Mec), a nAChR antagonist, significantly inhibited the Nic CT response in both WT and KO mice. The difference in the CT response between WT and KO mice in the absence of Mec (WT-KO) was not significantly different from the CT response in the presence of Mec ((WT+Mec)-(KO-Mec)). This indicates that WT and KO mice share the same nAChR distribution, and this difference represents the Nic response due to its interaction with T2Rs. At Nic concentrations <5 mM, nAChR and T2Rs each accounted for 50% of the total response to Nic, and at 10 mM Nic the nAChR component increased to 59% and the T2R component decreased to 41%. In KO mice, Mec combined with dihydro-beta-erythroidine (DHBE)inhibited the CT response to 10 and 20 mM Nic by 96% and 84.1%, respectively. This indicates that more than one type of nAChRs contribute to Nic CT response in WT and KO mice. In rats, Mec combined with DHBE inhibited the CT response to 10 mM Nic by 89%, indicating that in rats the T2R component represents only 11% of the total CT response to Nic. In rats, Mec, DHBE, and CP-601932 (a partial agonist of alph-3beta4* nAChR), inhibited CT responses to Nic ACh, and ETOH. CT responses to Nic and ETOH were also inhibited by topical lingual application of 8-chlorophenylthio (CPT)-cAMP and loading taste cells with [Ca2+]i by topical lingual application of ionomycin + CaCl2. In contrast, CT responses to Nic were enhanced after reducing taste cell [Ca2+]i by topical lingual application of BAPTA-AM. In patch clamp experiments, a subset of rat fungiform taste cells exposed to Nic responded with an increase in Mec-sensitive inward currents. In isolated circumvallate taste bud cells mRNA for alpha3, alpha4, alpha5, alpha6, alpha7, beta2, and beta4 were detected by real time RT-PCR. Immunohistochemical studies demonstrated the binding of specific alpha3, alpha4, alpha5, alpha6, beta2, and beta4 antibodies to fungiform and circumvallate mouse taste bud cells. In circumvallate taste buds obtained from TRPM5-GFP transgenic mice, alpha3 and beta4 antibodies demonstrated specific binding to a subset of TRPM5-postive taste cells. We conclude that multiple nAChRs expressed in TRPM5-positive cells serve as common receptors for the detection of TRPM5-independent bitter taste of Nic, ACh, and ETOH.

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P41 - Ric-8B is required for normal olfactory function.

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The olfactory system is able to discriminate a vast number of odorants. This ability derives from the existence of a large family of odorant receptors expressed in the cilia of the olfactory sensory neurons. Odorant receptors signal through the olfactory-specific G-protein (Golf), which stimulates adenylyl cyclase III to produce cAMP, which in turn triggers the opening of cyclic nucleotide gated channels and ultimately membrane depolarization. We have previously shown that Ric-8B, a guanine nucleotide exchange factor, interacts with Gαolf and is able to amplify odorant receptor signal transduction through Gαolf in vitro. In order to explore the function of Ric-8B in vivo, we generated a tissue specific knockout mouse by crossing OMP-Cre transgenic mice to Ric-8B floxed mice. Western blot analysis showed that knockout mice do not express Gαolf in the olfactory epithelium. These experiments also show that expression of OMP and Gγ13 in the olfactory epithelium are significantly reduced, when compared to wild type mice. Immunohistochemical experiments show that in knockout mice, the mature OSNs layer is reduced, and restricted to the apical layer of the olfactory epithelium. Finally, behavioral tests showed that knockout mice have a reduced ability to perform an odor-guided search task.

P42 - Olfactory and vomeronasal progenitor cell proliferation in Niemann-Pick type C1 disease after treatment with cyclodextrin.

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Niemann-Pick disease type C1 (NPC1) is a very rare neurovisceral lipid storage disorder characterized by a deficiency of the NPC1 gene function that leads to progressive neurodegeneration. Since olfactory impairment is one of the earliest symptoms in neurodegenerative disorders, we investigated the effect of defective NPC1 gene on olfactory and vomeronasal epithelia in an NPC1 mouse model. A combination of a substrate-reduction therapy (SRT) with miglustat and byproduct therapy (BPT) with cyclodextrin has been shown to delay the onset of neurological symptoms. Previous research confirmed severe morphological and immunohistochemical alterations in the olfactory system of NPC1(-/-) mutants compared with healthy controls (Hovakimyan et al., 2013). Here, we investigated the replacement capacity of the olfactory (OE) and vomeronasal

(VNO) epithelia in adult untreated NPC1(-/-) and NPC1 (+/+) animals by using two different therapy approaches: one group with a combination of SRT and BPT and one with cyclodextrin only. Using BrdU to label dividing cells, we detected for the OE a significant proliferation increase of 75%, SEM=10% in NPC1(-/-), 161%,SEM=11% in SRT/BPT NPC1(-/-)and 331%,SEM=7% in cyclodextrin NPC1 (-/-), indicating a high regenerative potential of olfactory basal cells in NPC1. Surprisingly, we also detected a massive therapy-induced proliferation in both treated control groups: 325%,SEM= 14% in SRT/BPT treated WT and 331%,SEM= 9% in cyclodextrin treated WT, compared with untreated controls. Preliminary results of the VNO also showed a similar proliferation increase of 181%,SD= 31% in NPC1(-/-) compared to untreated WT, and 224%,SD= 14% in SRT/BPT NPC1(-/-), respectively. Similar to the OE, we detected (1) a high regenerative potential of VNO basal cells and (2) a massive therapy- induced proliferation in both treated control WT groups compared with untreated controls. Further, we estimated the total number of mature olfactory receptor neurons (ORNs) in the main OE using Olfactory Marker Protein (OMP). Stereological analyses showed a dramatic decrease of OMP+ cells in the OE of NPC1(-/-) (47%,SEM=9%) and significantly higher rates in both treated NPC1(-/-) groups (SRT/BPT 68%,SEM=7%; cyclodextrin 83%,SEM=12%). The OMP+ rates do not completely reflect the highly increased proliferation rates of basal cells. Interestingly, cyclodextrin, administered alone, exhibited a proliferative activity of OE basal cells leading to increasing numbers of mature ORN in NPC1.

P43 - Orexin receptor 1 antagonist in anterior piriform cortex blocks aversive flavor-taste learning.

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Previous immunohistological and ibotenic lesions studies in our laboratory showed a role of anterior piriform cortex (aPC) in conditioned flavor preference (CFP). This type of learning develops a preference for a neutral flavor that is associated with a previously preferred taste. Orexin have been related with taste and odor learning (appetitive and aversive) and orexin OX1R receptors have been detected in piriform cortex. With this background, we investigated whether the inactivation of orexinergic receptors in the aPC prevents the acquisition of a flavor-taste learning induced by saccharin or whether it also affects flavor aversion learning (FAL) induced by quinine. In three acquisition sessions (six days) to develop flavor preference (Experiment 1) or aversion (Experiment 2), two doses (3 and 6 µg/0.5 µl) of the orexinergic antagonist SB-334867-A were bilaterally administered in the aPC immediately after a 15-min flavor intake period. The preference and aversion were tested on day 7 by two bottle-test sessions containing flavors used for CS + and CS – in a counterbalanced left-right position. Our results showed that SB-334867-A in the anterior piriform cortex blocks FAL, but does not block the capacity to acquire a flavor-taste preference learning. These findings appear to rule out that data obtained in FAL were attributable to any generalized or sensory (flavor) incapacity induced by SB-334867-A. Nonetheless, additional experiments may be warranted to examine the importance of the order of the presentation of the two procedures, CFP and FAL. Further studies are required to determine the functional relevance of the piriform cortex in taste learning. In the meantime, the present data appear to indicate that OX1R receptors in aPC play a role in the development of aversive flavor learning. aPC may function not as a primary olfactory region but rather as an association cortex necessary for flavor learning in which the hedonic value of the flavor is altered. These findings tentatively suggest that orexinergic fibers from the lateral hypothalamus to the aPC may provide an essential signal to reinforce the association between the stimuli (flavor and aversive taste) required for learning to take place.

P44 - A social chemosignal may reduce aggression in humans.

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Several main olfactory receptor types and subfamilies have been specifically implicated in rodent social chemosignaling, and these types and subfamilies are largely retained in humans as well. One such subfamily is the mammalian-specific OR37, whose subtypes A, B and C are activated by the long-chain aliphatic aldehydes pentadecanal, hexadecanal and heptadecanal, respectively. OR37 glomeruli are clustered in a small ventral domain of the olfactory bulb, a region implicated in processing socially relevant cues. Rodents release typical OR37 ligands in bodily secretions such as feces, and exposure to these ligands reduces stress responses in conspecifics. This has lead to the suggestion that OR37 ligands may act as promoters of social buffering (Klein et al., 2015). Humans similarly secrete these ligands, and hexadecanal for example can be found in human feces, breath and skin. With this in mind, we set out to test the hypothesis that hexadecanal (HEX) (C16H32O) will promote social buffering in humans, and thus reduce aggressive behavior. We conducted a double-blind between-subjects design experiment in which subjects were exposed to either HEX masked in Eugenol or to Eugenol alone. Subjects then engaged in social interaction that provokes aggression. Specifically, an ultimatum game played with a faux partner served as an aggression-evoking phase in which subjects encountered a frustrating refusal to cooperate that lead to financial loses. Then, the subjects were given the chance to discharge aggression in the form of a noise blast game (NBG) played against the same partner.

Subjects induced and received loud and unpleasant noises, the duration and volume of the noise served as measures of aggression. In addition, we collected self-ratings of mood, salivary testosterone, and the following psychophysiological measures: galvanic skin response (GSR), heart rate, respiration rate, ear pulse and skin temperature. Preliminary results (N=2) indicate that the subject exposed to HEX used shorter and less intense noises in compared to a control subject (noise duration mean HEX = 2726 ± 120.4 ms; control = 3875 ± 509.2 ms, t(32) = 2.197, P = 0.018, noise volume HEX = 2.588 ± 0.2111, control = 4.353 ± 0.4107, t(32) = 3.822, P = 0.0003). This reduced aggression was reflected in reduced variability in GSR (HEX GSR difference: 3.0676 ± 0.47 μS; control: 3.8149 ± 2.93 μS) and body temperature (HEX temperature difference: -0.0138 ± 0.04 C0; control difference: 0.328 ± 0.08 C0). Theses preliminary findings imply that HEX, a compound secreted in various human excretions, may act as a chemosignal to reduce aggression.

P45 - Ric-8B, an olfactory GEF, is essential for the development of the nervous system.

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Ric-8B is a guanine nucleotide exchange factor (GEF) expressed in mature olfactory sensory neurons in adult mice. We have previously shown that Ric-8B interacts with both Gαolf and Gγ13, two G protein subunits which are required for odorant signal transduction that occurs in the cilia of olfactory sensory neurons. In vitro, Ric-8B is able to amplify odorant receptor signaling through Gαolf; however, its physiological role remains unknown. To determine the role played by Ric-8B in vivo we generated a Ric-8B mutant mouse by using a gene trap approach. We found that, despite the limited distribution of Ric-8B gene expression in adult mice, Ric-8B homozygous mutants are not viable, and die around embryonic day 10.5. Mutant embryos are smaller and fail to close the neural tube at the cranial region. From embryonic day 8.5 to 10.5, Ric-8B gene expression is restricted to the neural folds of the cranial region and to the floor plate of the neural tube. We found that homozygous mutants have increased apoptosis in the cranial neural tube and head mesenchyme, which is possibly involved in the failure of the neural tube closure. In order to better understand the molecular function of Ric-8B in the development, we are analyzing the transcriptomes of wild type and mutant embryos.

P46 - Interactions of human nasal glutathione transferases with odorants.

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Odorant metabolizing enzymes (OME) and other proteins including odorant-binding proteins participate to a series of processes modulating the odor perception. These proteins are thought to respectively participate in odorant degradation or transport. The odorant metabolization may result in (i) the formation of new odorants (metabolites), (ii) changes in odorant bioavailability (iii) the receptor signal termination. Different OMEs as carboxylesterases or glutathione transferases (GSTs) have been shown to be involved in the mammalian olfactory process. GSTs are ubiquitous enzymes present in many tissues as liver, brain, olfactory mucosa or mucus. GSTs can be divided in six gene-independent family including the GSTA1 and GSTP1, both found in the human olfactory mucus. GSTs exhibits different enzymatic functions, including the conjugation of a variety of hydrophobic compounds to glutathione or isomerisation reaction, but these enzymes are also involved in molecules transport. We recently demonstrated in vitro the dual function of the human GSTA1 toward odorant molecules: odorant glutathione-conjugation and odorant transporter. Here, we deciphered the structure function relationship between GSTA1 and odorants. To reach this goal, we heterologously expressed human GSTA1 in E. coli. The recombinant protein was purified with purity greater than 99%. A large screen of odorants (different chemical families, such as: alcohol, ketone, aldehyde, acid molecules) interacting with GSTA1 was performed. The analysis was then refined to investigate, stereoselectivity (D, L limonene), chain length (fatty acid, ketone series), nature or the chemical function of the odorants structure in the interaction with GSTA1. Our work brings new elements to understand the binding mechanisms of GSTs, and their potential role in the olfactory peri-receptor events.

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P47 - Electrophoretic patterns of major urinary proteins (MUPs) in two inbred strains of laboratory mice resemble distinct 1D barcode.

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Major urinary proteins (MUPs) of the house mouse form a large group of highly polymorphic acidic isoforms with molecular masses of 18–20 kDa. MUPs are encoded by the Mup gene cluster, which consists of about 35 genes and pseudogenes and is mapped to chromosome 4. MUPs are considered as a key component of the mouse olfactory signature which

can provide all essential social information about individuality of donors (e.g. Churakov, Novikov, 2000; Hurst et al., 2001; Novikov et al., 2009). However, recently the prominent role of MUPs in formation an individual olfactory fingerprint (barcode) in wild-caught mice was called in question (Thoß et al., 2015). In this study performed on laboratory mice of two inbred strains we examined individual ontogenetic profiles of MUPs in both sexes and patterns of Mup genes expression in castrated males after testosterone treatment. Using one-dimensional gel electrophoresis we evaluate eight different bands (A-H) which formed genotype- and sex-specific MUP pattern in CBA/LacY and C57BL/6JY strains and in their F1 hybrids (CBAB6F1 and B6CBAF1). Quantitative evaluation of individual electrophoretic patterns (N=328) revealed that each genotype is characterized by quite specific combinations and relative ratios of just the same MUP isoforms. Further analysis separates phenotype cluster of MUPs into two distinct sub-groups (modules) of MUP isoforms. The first module consists of major fractions A,C,D, and E whose relative ratios show high stability during ontogenesis and thus can create genotype- and gender-specific olfactory images (Novikov et al., 2009, 2015). The second MUPs module consists of much more changeable minor isoforms B,F,G, and H. Taken together, these data give strong evidence that the differential and combinatorial gender-specific pattern of Mup genes expression in two common strains of laboratory mice successfully provided 1D «barcode» phenotype. We propose that various pheromonally active volatile compounds (VOCs) with differential binding capacities to different MUP isoforms (Kwak et al., 2012) represent «letters» of the chemical language in Mus musculus and, correspondingly, combinations of different MUPs efficiently organize these «letters» into distinct and readable for receiver «words» which encode sex, age, physiological state, hierarchical status, and genotype of donors. The obtained results can provide valuable insight into olfactory mechanisms of sexual recognition and mate choice in feral mice in order to pinpoint concrete Mup genes as a testable cause of mate preferences in hybrid zone between two European subspecies – Mus musculus musculus and Mus musculus domesticus (e.g. Smadja, Ganem, 2008; Bímová et al., 2011; Janoušek et al., 2012; Smadja et al., 2015).

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P48 - Does sickness behavior include changes in odor perception?

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Sickness behavior is a set of behavioral changes in response to infection and is elicited by proinflammatory cytokines. It is thought to redirect energy away from disadvantageous behaviors and toward an effective immune response. Anorexia, altered appetite, anhedonia, and social withdrawal are examples of reported sickness behaviors that could possibly affect olfactory perception during sickness. In a within-group design, 22 participants were injected with either saline (control) or lipopolyschararide (2.0 nanogram/kilogram body weight) to induce a transient systemic inflammation. For a few hours post-injection participants showed heightened levels of proinflammatory cytokines, tympanic temperature, and subjective sickness. At 1.5h post-injection, they evaluated 8 odors categorized as food (n=2), social (n=3) or other (n=3). Preliminary results indicate the food odors were rated as more disgusting as a function of systemic inflammation, suggesting that sickness behavior includes changes in olfactory perception.

P49 - Expression of butyrate receptor GPR43 in rats colon and the dietary fiber from raspberry.

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Introduction: Short chain fatty acids (SCFA) like, propionate and butyrate are the major molecules produced by the bacterial fermentation of dietary fiber (DF) in colon. Recently, the butyrate has been recently studied because is important to maintain colonic functions phisological and because it has been related with a protective effect in colorectal cancer, which is mainly, explained by its potential to regulate gene expression by inhibiting enzyme histonedeacetylase (HDAC). Several investigations shown that SCFA receptor GPR43 is involved insignal transduction mechanisms once they bind to ligands such as propionate and butyrate to generate different physiological effects in the colonocytes the mammals. Objective: Determine if dietary fiber consumption from raspberry (Rubus idaeus) containing a ratio of soluble-insoluble fiber 40/60, has a direct influence on the quantitative expression of butyrate-specific receptor GPR43. Methods: Wistar rats were fed with four different diets formulated at different concentrations of dietary fiber of 0, 5, 10 and 15% of dietary fiber from raspberry, respectively and the expression was determined with rt-pcr quantitative. Results and discussion: The results shown an increase in the expression of GPR43 (93.1%) when rats was fed with a 5% fiber diet, using β-actin as a reference gene. The results of this research will contribute to determinate the relation of diet (dietary fiber) with intestinal health for the purpose of expanding the knowledge of butyric acid on colonic functions physiological.

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P50 - Major Depression in the elderly is genuinely linked to olfactory impairments.

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Olfactory sensitivity is reduced in patients with Major Depressive Disorder (MDD). However, it has been discussed whether this olfactory dysfunction might be due to a cognitive decline accompanying MDD. In addition, olfactory functioning varies with the diagnostic subtype of affective disorders and age of participants. A highly selected sample of 11 elderly MDD patients (mean age: 63.3, SD: 11.1) with no concurrent mental disease was compared to a sample of 12 healthy controls (mean age: 69.2, SD: 4.5). None of the participants did show any signs of cognitive decline (as assessed by the "Cognitive Performance Test for Assessing Deficits of Memory and Attention", SKT and the "Mini Mental Status Exam", MMSE). Olfactory performance (sensitivity, identification, and discrimination) was assessed and odor ratings (intensity, familiarity, pleasantness, and unpleasantness) were obtained. Participants of both groups generally performed at a relatively low, but age corresponding level in the three olfactory tests. In addition, MDD patients showed a reduced olfactory sensitivity as compared to healthy controls ($p = 0.01$). The olfactory decline in MDD does not affect performances in odor identification ($p = 0.73$) or odor discrimination ($p = 0.87$). Odor ratings did not differ between groups (all ps > 0.10). To our knowledge, this is the first study demonstrating that the olfactory dysfunction in MDD is not secondary due to a cognitive dysfunction. The reduced olfactory sensitivity in MDD patients is a highly specific performance decline and can be shown independently of an age-related decline. However, the results are demonstrated with a relatively small sample of patients.

P51 - Odorant Binding Protein Biosensors for detection of drugs.

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Odorant binding proteins are small soluble proteins found in the chemosensory systems of mammals and insects. Because of their conformational stability they are of interest as biorecognition elements for new biosensors. Six wild type (WTs) recombinant Odorant Binding Proteins (OBPs) originated from Mosquito Anopheles gambiae (AgamOBPs) previously developed in our laboratory were selected, these AgamOBPs were AgamOBP1, AgamOBP4, AgamOBP5, AgamOBP19 and, AgamOBP47. These WT AgamOBPs were then screened for their ability to bind a selection of target drugs in solution using fluorescence based competitive binding assays. We then modified the binding pocket of AgamOBP1 using molecular biology techiques to produce mutant proteins with enhanced sensitivity and selectivity towards selected target drugs - Atropine, Cocaine, THC, Ephedrine, MDMA (Ecstasy) and Heroin. To test the responses of these proteins to analyte vapours, OBPs were immobilized on Quartz Crystal Microbalances (QCM) using a self-assembled monolayer. QCM measurements were carried out at 22%RH at 23 ºC; the baseline was established with clean air flowing at 0.1 L/minute. Saturated analyte vapours were sampled for 10 seconds. Repeated measurements were carried out over several months. The immobilised OBPs remained stable for many months and they were able to sensitively detect target analytes in the vapour phase. This opens a new approach to designing biosensors targeted to specific analytes.

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P52 - The influence of maternal body odors on preterm infant development.

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Preterm infants (PIs) have to undergo rapid neurodevelopment to qualify for discharge from hospitalization. Immediately after birth, the PI is hospitalized in a neonatal intensive care unit (NICU). This environment is strikingly different from womb ecology: light, sound and visual stimulation are abundant, and the PIs experience a great deal of stressful events mainly in the form of medical interventions. The consequences of such constant stress are associated with decreased neurodevelopment as well as down-regulation of homeostatic mechanisms via sympathetic nervous system activation. As early as the 28th week of gustation, the olfactory system of the fetus is mature enough to allow chemoreception. Exposure to different odors from this age and on alters PI behavior, reducing stress, pain and apnea. Several odors, including body odors (BO), are known as effectors of the parasympathetic nervous system (PNS). Shortly after birth, a newborn is able of recognizing its mother's BO, but the natural exposure of PI to its mother's BO is reduced significantly while in NICU. We hypothesize that long-term exposure of a PI to its mother's BO can reduce stress levels

through PNS activation and thus improve physiological status, eventually resulting in a shorter period of hospitalization (POH). To test this hypothesis, we are currently exposing PIs to maternal odors. We asked mothers of PIs to wear a breast pad in direct contact with the body for a 24-hour period, while keeping their regular diet routine. Pads were then placed inside the incubator of the matched PI for a 24-hour period. This process is repeated for 5 consecutive days per week, until the PI is discharged from NICU. We make use of the routine hospital medical checks for respiratory rate, heart rate, blood pressure, and blood-oxygen saturation. In addition, we collect metrics such as amount and type of medical interventions, POH and weight gain rate. Sleep quality measurements are evaluated by actigraphy. In order to estimate effects on PNS we measure salivary cortisol levels. Samples of breast pads are analyzed by GCMS in order to identify volatile components that may be the mediators affecting the PIs. The results of this ongoing effort will be presented.

P53 - Complexity of voltage-gated sodium channel distribution in the mouse olfactory subsystems.

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Detailed knowledge about the distribution of voltage-gated sodium channels (Nav) employed by the olfactory system is an essential prerequisite to understand their functional contribution to action potential generation, propagation, and olfactory signal transmission. We previously showed that Nav1.7 is the predominant isoform expressed in the main olfactory system and that this channel plays an essential role in odor perception. Deletion of Nav1.7 in mouse olfactory sensory neurons (OSNs) causes anosmia due to a lack of Nav1.7 in OSN axons and presynaptic boutons, precluding synaptic transfer in the main olfactory bulb glomeruli (Weiss, Pyrski et al., Nature, 2011). Interestingly, Nav1.3, a second isoform we identified in the olfactory epithelium cannot compensate the lack of Nav1.7, because of its absence in the OSNs presynaptic boutons, exemplifying that subcellular localization impacts function. The mouse olfactory system consists of multiple subsystems with distinct functions and signal transduction mechanisms, but it is unknown whether each subsystem employs a unique set of sodium channels or whether all subsystems share the same channels. Here we address the cellular and subcellular distribution of different sodium channel isoforms in the main and accessory olfactory systems, and in the Grueneberg ganglion of adult mice using a combination of quantitative real time RT-PCR and fluorescence immunohistochemistry. Our PCR results show that both MOE and VNO contain the isoforms Nav1.1, Nav1.2, Nav1.3, Nav1.5, Nav1.6, and Nav1.7. We find that Nav1.7 and Nav1.3 represent the most abundant isoforms in both MOE and VNO. Consistent with the PCR results, strong immunoreactivity for Nav1.7 and Nav1.3 is present in axonal processes of OSNs and VSNs. Nav1.7 but not Nav1.3 is also present in a subpopulation of microvillous cells in the MOE. Interestingly, we also detect both isoforms in neurons of the Grueneberg ganglion. Subsequent study of Nav1.2 and Nav1.6 reveals a more complex distribution of these isoforms. In the MOE, Nav1.2 immunoreactivity is primarily detected in a subpopulation of microvillous cells. In the VNO, immunoreactivity for Nav1.2 and Nav1.6 is restricted to VSN somata, with Nav1.2 primarily located in the somata of basal VSNs, while axons were devoid of staining for both isoforms. Taken together these results suggest that Nav1.3 and Nav1.7 are of general importance for electrical conductance in the sensory neurons of all three subsystems. The subcellular localization of Nav1.3 implies that this isoform primarily contributes to signal propagation, while Nav1.7 in addition contributes to signal transmission. By contrast, Nav1.2 and Nav1.6 define a small subsystem and their somatic localization indicates a role in VSN action potential generation.

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P54 - Factoring for individual component intensity in predictions of perceptual similarity of odorant mixtures.

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A major goal in olfaction research is the prediction of odor perception from odor structure. We and others have made some meaningful steps in this direction, such that we can now predict aspects like odorant pleasantness and pairwise odorant similarity from odorant structure alone. Typical correlations between predicted and actual odorant pleasantness are $r = 0.7$, $p < 0.001$ (Pearson correlation), and between predicted and actual odorant similarity $r = 0.8$, $p < 0.0001$. Moreover, these results hold for odorant mixtures made of many odorant component molecules. However, whereas odors in the real world consist of molecular mixtures of unequal intensities (that is, one molecule may be concentrated/intense in the mixture and others dilute/mild), all of the above lab work was conducted in mixtures of equated intensity. That is, mixtures where all molecular components were first equated for perceived intensity. Here we set out to test the hypothesis that individual component intensity will influence the perception of the mixture. To perform this, we estimate the impact of unequal component intensity on predictions of mixture similarity. We chose 44 different odorant molecules to prepare 14 odorant mixtures ranging in number of components between 4 and 10. Here, in contrast to our previous efforts with identical component mixtures, we did not first use dilutions to equate component intensity. Thus, these mixtures reflected realistic natural odorants. We

next asked 24 subjects (16 F, ages 22–37) to rate similarity of 95 such mixture pairs. Each pair was rated twice. Finally, we estimated the structural characteristics of each mixture using DRAGON analytical chemistry software, and applied our previously developed algorithms for predicting perceptual similarity from odorant structure to this newly acquired data. Whereas in mixtures with components equated for intensity the correlation between predicted and actual perceptual similarity was $r = 0.78$, $p < 0.0001$, in this data where components were not equated for perceptual intensity, the prediction power dropped to $r = 0.41$, $p = 0.003$. This significantly lower correlation ($Z > 4$, $p < 0.001$) implies that consistent with our hypothesis, component intensity influences mixture perception. In order to improve prediction power for mixtures with components of unequal intensity, we have used the new data to add an intensity factor to our algorithms. Our results indicate that individual component intensity factor should be added to the prediction of mixtures similarity according to a psychometric function. It allowed us to predict perceptual similarity of mixtures with components not equated for intensity at a correlation of $r = 0.69$, $p < 0.0001$ between predicted and actual similarity. We are now conducting an experiment with novel mixtures in order to validate the new algorithm.

P55 - Linking olfactory perception with the micro-biome.

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The human olfactory system depends on hundreds of different kinds of receptors with different levels of expression in each person. Moreover, these receptors are influenced by both genetic and epigenetic factors. This variety of combinations allows, in principal, that each and every one of us will have a unique nose. Recent work in our lab has revealed a complex picture: whereas humans are grossly similar in their olfactory perception, the use of a very sensitive perceptual test revealed highly unique perceptual profiles we termed olfactory fingerprints. In addition to genetic variability, the human body contains billons of bacteria that populate the gut, mouth, nose and more, also known as the micro-biome. Age, diet, genetics, health condition and environment influence each person's micro-biome. The micro-biome takes part in a variety of processes such as metabolism, reproduction and defense. The micro-biome is also correlated with various diseases, which in turn impact the olfactory system. Moreover, the micro-biome can affect mood, which can also impact the olfactory system. Given these links, we set out to test the hypothesis that the micro-biome influences olfactory perception. We measured perception using the olfactory fingerprint resulting from 22 odorants rated along 23 descriptors. We altered micro-biome using probiotics for 10 days (vivo mix, 1 portion per day). So far, only one subject has completed the entire experiment (M, age 30). The correlation between this person's pre-probiotics and post-probiotics olfactory fingerprints was $r = 0.73$. Given the expected population correlation of $r = 0.58 \pm 0.21$ for refingerprinting over time, this very initial results ($n = 1$) implies no sign of altered olfactory perception following probiotics.

P56

Abstract withdrawn.

P57 - Local postsynaptic sodium channel activation in dendritic spines of olfactory bulb granule cells.

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Dendritic spines are considered to have the ability to operate as independent signaling units. However the conductances involved in spine restricted signal generation remain speculative. Here we use the reciprocal granule cell (GC)/mitral cell (MC) synapse in the olfactory bulb (OB) to study local signaling in GC spines. In order to mimic a unitary input from the MC to the GC side we performed two-photon uncaging (TPU) of glutamate at single GC spines and used the spine calcium signals as well as the uncaging evoked EPSPs (uEPSPs) recorded at the GC soma as a read-out. We show that blocking Navs with 500 nM TTX resulted in a strong reduction of $(\Delta F/F)$TPU in most spines (to 0.63 ± 0.20 of control, mean ± S.D., $n = 34$). uEPSP amplitudes were only slightly decreased (0.88 ± 0.30 of control, $P < 0.05$) whereas the rise time became substantially slower and the duration substantially longer. Notably, the magnitude of the blocking effect of TTX on $(\Delta F/F)$TPU was highly correlated with the magnitude of the TTX-induced increase in rise time across experiments ($P < 0.005$ for both). We hypothesized that the extra depolarization provided by Navs boosts Ca2+ entry mainly via high voltage activated calcium channels (HVACCs). Thus we blocked N/P/Q type Ca2+ channels with ω-conotoxin MVIIC (CTX), which decreased $(\Delta F/F)$ TPU to 0.67 ± 0.19 of control ($n = 24$). CTX applied after TTX reduced $(\Delta F/F)$TPU only marginally ($n = 14$), similar to the reverse experiment (TTX after CTX;($n = 8$). Thus HVACC activation is the main source of Nav-induced Ca2+ entry. The blockade of other known postsynaptic calcium sources (NMDA receptors, internal stores and low voltage activated T-type calcium channels) did not occlude the effects of TTX. In turn, blockade of NMDARs post TTX application still had a considerable effect on $(\Delta F/F)$ TPU (to 0.11 ± 0.06 of control), indicating that NMDAR-mediated Ca2+ entry is not boosted by Navs. Next we investigated

the contribution of various types of K+ channels which are likely to get activated during Nav-mediated depolarization. Although blockade of Kv4 and Ca2+-activated K channels did not interfere with (ΔF/F)TPU and uEPSPs, a NEURON model supports the indirect evidence of a slowly repolarizing current that is Nav-activated and fits the properties of delayed-rectifier potassium channels (KDR). Our results suggest that the local Nav mediated mechanism may endow the olfactory bulb with an extra mode of computation that serves to fine-tune the primary representation of olfactory sensory stimuli. The observed acceleration of EPSPs by Navs may contribute to precise timing of GC output, e.g. in the context of fast sensory-evoked network oscillations.

P58 - Hierarchical Deconstruction of Olfactory Sensory Neurons: from Whole Organ to Single-Cell RNA-seq.

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The mouse olfactory mucosa is a complex chemosensory tissue composed of multiple cell types, neuronal and non-neuronal. We have here applied RNA-seq hierarchically, starting with crude tissue samples dissected from the nose, proceeding to flow-cytometrically sorted pools of mature olfactory sensory neurons (OSNs), and finally arriving at single mature OSNs. We show that 98.9% of intact olfactory receptor (OR) genes are expressed in mature OSNs, and uncover a hitherto unknown bipartition among mature OSNs. We find that 19 of 21 single mature OSNs each express a single intact OR gene abundantly, consistent with the one neuron-one receptor rule. Monoallelic expression of this abundantly expressed OR gene is extremely tight. The remaining two single mature OSNs appear to be examples of type B Trpc2+ cells in the main olfactory epithelium, which we here establish as a neuronal cell type that is fundamentally distinct from canonical OSNs.

P59 - Tracing of mono- and polysynaptic afferent connections between the main olfactory bulb and higher-order brain regions in the mouse.

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Processing of odors in the main olfactory bulb (MOB) is modulated by higher brain afferents depending on the internal state, motivation, memory and emotions. For example satiety or hunger are known to change the perception of food odors. To shed light on this modulation a greater understanding of the underlying circuitry is required. To this aim we conducted tracing experiments in mice. First, stereotaxic injections of monosynaptic retrograde DiI and choleratoxin subunit B (CTb) into the dorsal olfactory bulb (focusing on the granular layer) were performed. In a second approach, we injected the pseudorabies virus 152 (PRV152) (kindly provided by Prof L. Enquist; Princeton University). As a neuronal tracer, this neurotropic virus can spread in synaptically connected neurons, dissecting the entire circuitry. The temporal analysis of the viral distribution allows to determine the number of synapses crossed. Both DiI and CTb confirm all the main centrifugal afferents to the MOB which were previously described in the literature. Thus, except the olfactory tubercle, all regions belonging to the olfactory primary cortex were labeled. Moreover, in the piriform cortex, labeling was mainly located in its dorsal part, confirming the topographical anatomical organization of cortico-bulbar projections. Direct projections arising from orexinergic neurons in the lateral hypothalamus were also observed. Regarding polysynaptic tracing, mice were sacrificed one, two and three days after PRV152 injections. After one day, only some of the direct neuromodulatory afferents were labelled. Thus, the locus coeruleus, which has a very caudal location in the brain close to the forth ventricle, showed already staining. In contrast, other neuromodulatory afferents (arising from raphe nuclei, ventral tegmental area, basal forebrain) were not labeled at this stage. Two days after injection, comparison with DiI/CTb results indicate that all the first-order connections were labeled. At this stage, second-order projections started to appear (for example, the midline thalamic nuclei as reuniens and rhomboid nuclei). Lastly, after three days, an extensive brain labeling occurred although some brain regions still showed a lack of staining. In conclusion, using DiI/CTb tracers that do not cross synapses allow to stain all primary afferents. In contrast, viral tracer migration depends on timing as well as number of connecting synapses. Thus, labeling occurring already one day after injection indicates a strong connection. Accordingly, our results suggest that the stained cells in the locus coeruleus send strong projections to the MOB. Furthermore this method allows to follow up the circuits involved in olfactory modulation. Data analysis of all the other labeled regions, focusing on hypothalamic nuclei and brain areas involved in arousal and food intake, are in progress.

P60 - Multimodal priming effects of facial expressions as visual stimuli on olfactory perception and cognition - an fMRI study.

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Integrating multimodal information into a common percept appears to be vital to react to different stimuli and interact with the environment. Especially, in the social environment not only visual, but also auditory and olfactory information can change the course of an interaction. The perception and processing of emotions, in form of facial expressions appears to be substantial and are known to be altered by emotional stimuli from an other modality as contextual information, but it is less examined, whether it possible that an emotional face expression can change the perception of an other emotional stimuli. Thus, a main interest of this study is to examine, if facial expressions have an influence on the perception of odors. 17 subjects (7 male and 10 female; mean age: 23.64 years ± 2.34; range: 18–28) participated in the current fMRI-study. 162 faces (54 disgusted, 54 neutral and 54 happy) from The Bochum Emotional Stimulus Set database and 12 odors were used as stimuli material. A cross-modal priming task with emotional faces (disgusted, neutral or happy) as primes and odors (positive and negative) as targets was used to investigate social influences on odor perception and to analyze the neural correlates. A MRI compatible multichannel (24 channels) olfactometer for presenting many odors in fast intervals was used. Behavioral results show significant effects of odor valence with pleasant odors being rated as more pleasant than unpleasant odors. Faces after seeing an disgusted face odors were rated as more unpleasant than after seeing an happy face. The results of the fMRI data shows significant main effects of odor valence and faces for various brain areas, which are both modulated by an significant interaction. For incongruent stimuli versus congruent stimuli the bilateral amygdalae, hippocampus and piriform cortices show a significant increase in activation. For congruent versus incongruent stimuli the Wernicke's area and the fusiform gyrus show a significant increase of activation. The behavioral results as well as the fMRI data suggest that perception of odors can be primed by social stimuli. Seeing emotional faces can shift the valence perception of an odor towards the emotional quality of that facial expression. Increased activations of incongruent stimuli reflect the conflict evoked by unfulfilled expectations by the odor not fitting to the social cue. This leads to an increased attention towards the odor object and its valence. Thus for example in occupation environments, in which an influence of odors on concentration and health is often discussed, not only the features of the odor can lead to nuisance but also the context, in this case the social context, in which the odor is perceived. For future studies it would be interesting to investigate the influences of odor features like concentration on the perception and processing of olfactory stimuli with and without a social context and the involved neural substrates.

P61 - Olfactory receptors of Drosophila are sensitive to molecular volume of odorants.

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Which properties of a molecule define its odor? This is a basic question of olfaction, yet to be answered. Human olfactory system has a repertoire of about 350 olfactory receptors. Molecules bind to them with different affinities and activate them with different efficacies, resulting in a combinatorial code that identifies odorants. We hypothesized that the binding affinity between a pair of odorant-receptor is affected by their relative sizes. The affinity can reaches its maximum if molecular volume of an odorant matches volume of a receptor's binding-pocket and it reaches zero if the sizes are too different, obscuring the effect of other molecular properties. We formulated this mathematically and verified it on Data of Drosophila (DoOR database), and predicted the volume and the structural flexibility of each receptor's binding-site, which are significantly different among receptors. This provides a reason for differences in smell among similar molecules of different sizes.

P62 - Modeling and experimental verification of ligand-receptor interaction in a high affinity cadaverine receptor with an unusual bifunctional ligand requirement.

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Olfaction is the sense of smell, which regulates a vast number of behaviours. Fish are equipped with only one olfactory organ containing both ciliated and microvillus OSNs (Olfactory Sensory Neurons) that project their axons to the OB (Olfactory Bulb). Five different families of vertebrate olfactory receptor gene families are known: odorant (ORs), vomeronasal (V1Rs and V2Rs), formyl peptide receptors (FPR's) and Trace amine-associated (TAARs) receptors (Korsching, 2008; Hussain et al., 2009). In the framework of a ligand search for TAAR receptors, recently in lab, we identified high affinity receptor for cadaverine, TAAR13c. Structure-activity analysis indicates TAAR13c to be a general diamine sensor, with pronounced selectivity for odd chains of medium length. TAAR genes segregate into 3 classes. We performed structural modeling together with intra-family sequence alignments, which led to the discovery of a potential candidate cadaverine-binding motif. It consists of an aspartate in the third transmembrane region and a tryptophan in the seventh transmembrane region. We are now interested to elucidate the structural dynamics of binding of cadaverine to TAAR13c by structural docking. The first step was building the structure for TAAR13c by homology and ab initio algorithm. The structure prediction reveals a cleft, which can be assumed as the binding pocket of this receptor. Inside of this potential binding pocket are D112 and W269 located, which belong to the classical aminergic-binding motif (figure 1.2). In close proximity and in the same plane there was another aspartate residue (D202) found (figure 1.2). It is conceivable that D112 and D202 interact with the positively charged amine groups of the di-amines. Based on this, we propose a model

of the ligand binding of TAAR13c whereby D112 and D202 bind the two positively charged amine groups via ionic interaction and W269 might stabilize the non-polar carbon chain. In order to test this hypothetical binding motif we mutated these positions to amino acids exhibiting a different charge or different chain lengths (Table1.3.) The mutated Taar13c ORF encoding N terminal Rho tag was cloned in pcDNA3.1 (-). We will use these plasmids to express the mutated TAAR13c ORFs in HEK cells and test the ligand binding by visualization of the intracellular Ca2+ concentration. Based on the outcome, we would perform further mutations.

P63 - Modulation of sweet taste by umami compounds via sweet taste receptor.

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Although the five basic taste qualities — sweet, sour, bitter, salty and umami — can be recognized by the respective gustatory system, interactions between these taste qualities are often experienced when food is consumed. Specifically, the umami taste has been investigated in terms of whether it enhances or reduces the other taste modalities. These studies, however, are based on individual perception and not on a molecular level. In this study we investigated umami-sweet taste interactions using umami compounds including monosodium glutamate (MSG), 5'-mononucleotides and glutamyl-dipeptides, glutamate-glutamate (Glu-Glu) and glutamate-aspartic acid (Glu-Asp), in human sweet taste receptor hT1R2/hT1R3-expressing cells. The sensitivity of sucrose to hT1R2/hT1R3 was significantly attenuated by MSG and umami active peptides but not by umami active nucleotides. Inhibition of sweet receptor activation by MSG and glutamyl peptides is obvious when sweet receptors are activated by sweeteners that target the extracellular domain (ECD) of T1R2, such as sucrose and acesulfame K, but not by cyclamate, which interact with the T1R3 transmembrane domain (TMD). Application of umami compounds with lactisole, inhibitory drugs that target T1R3, exerted a more severe inhibitory effect. The inhibition was also observed with F778A sweet receptor mutant, which have the defect in function of T1R3 TMD. These results suggest that umami peptides affect sweet taste receptors and this interaction prevents sweet receptor agonists from binding to the T1R2 ECD in an allosteric manner, not to the T1R3. This is the first report to define the interaction between umami and sweet taste receptors.

P64

Abstract withdrawn.

P65 - A new measure of odor complexity is also applicable across senses.

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We defined a new measure of odorants based on the amount of variance in answers to questions about them. Our definition is based on the intuition that odorants (and other stimuli) will vary in the amount of variance they invoke. For example, answers to questions about a red square will be more consistent than questions about a Jackson Pollock painting. Likewise in olfaction, some odorants consistently invoke more variance than others. We call such variance-inducing odorants complex or intricate odors. As a consequence of our definition, our measure is also performance based (as opposed to being based on assessment) and can be applied across modalities. We used three olfactory experiments to confirm that our measure is well defined and robust. In addition a vision experiment showed that our measure can indeed be applied to other senses consistently. Next weset out to test if our measure is correlated with well established measures of sensory processing. We compared our measure of the intricacy of visual stimuli with their masking effectiveness in an Rapid Serial Visual Presentation experiment. The result was that our measure is correlated at $r=0.75$ $p<0.002$ to masking effectiveness. This shows that our measure is not only well defined but also that it could be relevant to many different phenomenon and open to rich interpretation.

P66 - Morpho-functional study of the ovipositor sensilla in Drosophila suzukii.

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The aim of the study was to examine the presence and the specific sensitivity of gustatory sensilla located in the ovipositor of Drosophila suzukii, mediating the search for suitable oviposition sites, by means of a morphological, electrophysiological and behavioural approach. The scanning electron microscopy (SEM) analysis showed that the ovipositor surface in D. suzukii presents three different types of sensilla, among which 10 uniporous sensilla (5 per each valve). The electrophysiological recordings from these sensilla showed that they are sensitive to sugars (sucrose, glucose, fructose), bitter compounds (caffeine, nicotine) and ascorbic acid. Finally, behavioural trials showed that the number of eggs laid on the sugar substrates was significantly different from both bitter or acid substrates. In conclusion, morphological, electrophysiological and behavioral results suggest for the first time, in a Drosophila species, a chemosensory role for the ovipositor sensilla and their possible involvement in the choice of the oviposition sites.

P67 - Chemosensory cholinergic signaling network in the thymic medullary epithelium.

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Cholinergic signaling influences T cell maturation, and acetylcholine is endogenously synthesized in the thymus. Utilizing a reporter mouse strain that expresses GFP under the promoter of the acetylcholine synthesizing enzyme, choline acetyltransferase (ChAT), we detected cholinergic cells in the thymic medulla. Additional use of another reporter mouse strain (Tas2r131-tauGFP) and immunohistochemistry show that ChAT-positive cells co-express the bitter taste receptor Tas2r131 and the components of taste signaling cascade alpha-gustducin, phospholipase C beta 2 and transient receptor potential melastatin-like subtype 5 channel (TRPM5). These thymic cholinergic chemosensory cells are different from the stellate medullary thymic epithelial cells (mTECs) involved in intrathymic negative selection of thymocytes in that they do not express autoimmune regulator (AIRE) and express cortical (8/18) instead of medullary (5/14) keratins. They are not approached by cholinoceptive sensory nerve fibers. Instead, they are in proximity to terminally differentiated (keratin 10-positive, Hassall-like bodies) mTECs carrying nicotinic acetylcholine receptors (alpha 3-subunit). In human newborn thymus, these cells closely surround or are integrated in the outer layer of the Hassall's corpuscles. Similar cells in mucosal surfaces have been associated with detection of bacterial products. Hence, we quantified thymic mRNA expression of an array of genes involved in cholinergic and chemosensory transmission in streptococcal pneumonia-infected mice, which revealed 6-9fold up-regulation of TRPM5 and alpha-gustducin. In conclusion, we identified a novel chemosensory cholinergic cell type in the thymic medulla and hypothesize that there is a paracrine acetylcholine signaling between these cells and Hassall's corpuscles, and that this signaling plays a role in bacterial pathogen detection and defense.

P68 - Activation of the mouse OR37 subsystem coincides with a reduction of novel environment-induced activity within the paraventricular nucleus of the hypothalamus.

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Within the main olfactory system of mammals, a unique subsystem exists comprised of sensory neurons expressing odorant receptors of the OR37 subfamily. These receptors are exclusive for mammals and highly conserved across species. The mouse OR37 receptor subtypes A, B and C were shown to be activated by the long-chain aliphatic aldehydes penta-, hexa- and heptadecanal, respectively. The search for biological sources of these compounds now shows that bodily secretions from conspecifics activate the OR37A, B and C glomerulus. At the same time the activity of cells in a target region of projection neurons from OR37 glomeruli, the paraventricular nucleus of the hypothalamus (PVN), is reduced compared to controls (clean test box). The large number of activated cells in the PVN of mice that are placed into a clean test box are corticotropin-releasing hormone cells, indicating an induction of the stress axis due to the novel environment. The much lower number of activated cells of mice in a box enriched with bodily secretions from conspecifics indicates a reduced stress response. Since bodily secretions from conspecifics activate the OR37 system and simultaneously reduce stress-induced activation of the PVN, it was tested whether the ligands for OR37 receptors can induce this effect. Indeed, a similarly reduced activity in the PVN is found in mice kept in a clean test box and exposed to a mixture of the OR37 ligands delivered via an air stream. These data indicate that the OR37 system may play a role in mediating a phenomenon called social buffering.

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P69 - Ancestral amphibian V2Rs are expressed in the main olfactory epithelium.

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The sense of smell helps animal species to evade predators, localize prey and recognize viable mates. In mammals olfactory receptor families are segregated into different olfactory organs, main olfactory epithelium (MOE) and vomeronasal organ (VNO). In contrast, teleost fish olfactory receptor families are intermingled in a single sensory surface. To what extent such differences influence the coding and discrimination abilities of the respective olfactory systems is unclear, and the evolutionary path toward such segregation is unknown. The analysis of amphibians, which are early diverging tetrapods compared with mammals, may shed light on this transition from shared sensory surface to segregated subsystems. For this study, we focused on the V2R (vomeronasal type 2 receptor) gene family, by thorough datamining we showed the Xenopus V2R family to encompass nearly

500 genes in total. A phylogenetic analysis led to the identification of three distinct subdivisions in the largest group of V2R genes (A1, A2, A3). We used this sequence information to clone several Xenopus laevis V2R gene representatives of above mentioned subdivisions. Xenopus laevis (African clawed frog), is the system of choice for physiological studies of the amphibian olfactory system and furthermore, a close relative of Xenopus tropicalis. We report here that to our surprise, several V2R genes were expressed exclusively in the MOE, and not in the VNO. These genes occupied basal positions in the phylogenetic tree, whereas late diverging V2R genes were exclusively expressed in the VNO. Moreover, within the MOE V2R genes are expressed in a basal zone, partially overlapping, but clearly distinct from an apical zone of OMP and odorant receptor-expressing cells. 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.

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P70 - The extremely broad odorant response profile of mouse olfactory sensory neurons expressing the odorant receptor MOR256-17 includes TAAR ligands.

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Mouse olfaction relies on 1100 odorant receptors (ORs). A typical OR has a narrow response profile when tested with a panel of odorants, and but a few ORs are broadly responsive. Here, using patch-clamp recordings in an intact olfactory epithelium preparation, we have recorded odorant-induced currents from two populations of olfactory sensory neurons (OSNs) tagged with GFP, in the mouse strains SR1-IRES-tauGFP and MOR256-17-IRES-tauGFP. We find that MOR256-17+ OSNs have an extremely broad responsive profile, spanning several, dissimilar chemical groups. MOR256-17+ OSNs responded to 31 from the 35 chemical compounds tested, whereas SR1+ OSNs responded to 10 of these compounds. All compounds that stimulate SR1+ OSNs also stimulate MOR256-17+ OSNs. Interestingly, we also find that MOR256-17+ OSNs respond to amines that are conventionally thought of as TAARs ligands.

P71 - The Role of Notch Signaling in Olfactory Bulb Circuit Maintenance.

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The mammalian brain has a remarkable capacity for continued neurogenesis throughout life. Every day, thousands of adult-born neurons are generated in the subventricular zone and migrate anteriorly where they eventually integrate into the existing neuronal circuitry of the olfactory bulb (OB). During this process, only fifty percent of adult-born neurons will survive long-term while the rest are eliminated through apoptosis. This dramatic reduction suggests that certain selection events act on these neurons to determine which ones will be integrated. In order to find molecular mechanisms that regulate this selection, we performed a microarray analysis on mutants that we have previously shown have deficits in adult-born neuron circuit integration and found that Notch signaling is altered between mutants and control animals. Using a Notch reporter mouse, we found that Notch signaling is highly active in the OB. Detailed analysis of these reporter animals revealed that increased Notch levels are correlated with increasing maturity of adult-born neurons. Therefore we hypothesize that Notch signaling is involved in the maintenance of olfactory bulb circuits by promoting adult-born neuron integration and neuronal function. By investigating the function of Notch in the OB, we intend to find novel functions for Notch signaling in the regulation of neuronal plasticity and circuit maintenance.

P72 - Feedforward Excitation Entrains Oscillatory Activity in a Subpopulation of Mitral Cells in the Accessory Olfactory Bulb.

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The accessory olfactory system is a key component in rodent conspecific chemical communication. However, coding strategies within the involved brain areas - the accessory olfactory bulb (AOB), the 'vomeronasal' amygdala and the hypothalamus - are poorly understood. In the AOB, the first stage of information processing in the mouse vomeronasal pathway, mitral cells (MCs) - the AOB's projection neurons - receive sensory input from vomeronasal neurons. This sensory information is processed in the AOB network before it is relayed to third-order nuclei. A subpopulation of MCs exhibits slow oscillatory discharge that persists upon pharmacological inhibition of fast synaptic transmission. Here, we identify an excitatory circuit within the AOB network that entrains oscillatory activity in a second MC subpopulation. Using patch clamp recordings from MCs in acute AOB tissue slices, we investigate the mechanisms underlying oscillatory entrainment. Entrained MCs display periodically increased excitatory synaptic input that correlates with their respective rhythmic discharge patterns. Block of fast glutamatergic synaptic transmission reveals that entrainment depends on an intact glutamatergic network. Ongoing experiments aim to identify the detailed mechanisms of MC entrainment and the role of slow rhythmic activity in AOB information processing.

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P73 - Physiological Mechanism of Mating State-Dependent Detection of Polyamines.

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Animals undergo dietary transitions depending on their changing metabolic needs. In humans, pregnant females often experience modulations in their food preference possibly due to their changing needs. Odors and tastes are the main modalities for the evaluation of food. Using the vinegar fly Drosophila melanogaster, we have addressed whether and how gravidity affects the preference and processing of odors and tastes. The female vinegar fly prefers to lay its eggs onto, or in close proximity of a rotting fruit to provide optimal conditions for her developing progeny. Rotting or decaying fruits produce a significant amount of polyamines. Protein decomposition and bacterial metabolism are the main sources of the polyamine-based pungent smell of rotting organic compounds. In addition to being the signature odor of decaying organic material, polyamines are essential in all living cells for cell proliferation, differentiation, and embryonic development across species. Therefore, polyamines may not only be indicators of potential food or egg laying sites, but also important nutrients for gravid females. We found that flies are highly attracted to volatile polyamines at concentrations found in rotting fruit. We show that flies detect volatile polyamines using a specific ionotropic receptor, IR41a, located on their antenna. Importantly, although both male and female flies are attracted to polyamines, female flies shift their preference to significantly higher polyamine concentrations upon mating, consistent with their increased need in polyamines and protein. Using a combination of behavioral analysis and in vivo calcium imaging, we show that the G-protein coupled receptor SPR (sex peptide receptor) and its neuropeptide ligand MIPs (myoinhibitory peptides) play the key role in the modulation of the preference behavior by tuning the sensitivity of IR41a olfactory neurons to higher polyamine concentrations upon mating. Remarkably, SPR signaling is required directly in the olfactory neuron. Together, our data elucidates how the detection and hence the preference for the same chemosensory input can be modulated depending on changing needs of a gravid female.

P74 - Somatostatin-IRES-Cre Mice: knock-in and knock-out?

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The neuropeptide somatostatin (SOM) is largely expressed in mouse brain. In many regions such as cortex or hippocampus, it is co-expressed with gamma-aminobutyric acid (GABA) and calretinin. In the olfactory bulb, however, one-half of SOM-immunoreactive (SOM-ir) GABAergic neurons are parvalbumin-ir, revealing an atypical neurochemical profile when compared to SOM-ir GABAergic interneurons of other forebrain regions. Somatostatin-IRES-Cre (SOM-Cre) mouse strains are increasingly used to unravel the physiology of somatostatin-containing neurons in the brain. However, while knock-in targeting strategy greatly improved Cre-Lox system fidelity, recent reports indicate that genomic insertion of Cre construction per se can markedly affect physiological function, depending on the genomic organization of the targeted gene. Since the SOM-Cre construction is targeted to the 3'UTR region of the sst gene that we recently described as highly evolutionary with potential regulatory function, we characterized the molecular and physiological impact of the Cre transgene on endogenous SOM tone. By RT-qPCR, SOM mRNA in the cortex of homozygous and heterozygous SOM-Cre mice amounted to 12 % and 62% of wild-type mice, respectively. In contrast, neuropeptide Y and SST1-4 mRNA levels were not modified. By radioimmunoassay, SOM peptide concentrations in the olfactory bulb, cortex, hypothalamus and cerebellum of homozygous SOM-Cre mice amounted to less than 10% of wild type mice. SOM-Cre mice also displayed an exaggerated corticosterone response to acute restraint stress though it did not reach the high levels observed in sst -/- mice. Thus, the demonstration that SOM peptide levels and SOM-related physiological responses are impacted needs to be taken into account when using the SOM-Cre strain. especially because transgene insertion may affect mRNA stability and disrupt given central and peripheral SOM regulations. It is of particular interest though that heterozygous SOM-Cre mice display decreased brain SOM concentration and increased stress responses of the same magnitude than those observed in clinical mood and cognitive symptoms across neurological and psychiatric disorders.

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P75 - Neonatal Exposures to Cat Odors in the House Mouse Produce Sensitization and Habituation to Target Signals at the Behavioral Level but Not at the Hormonal.

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Chemosensory detection is a very important aspect for predator avoidance strategy for many mammals including the house

mouse. Odors from carnivores may elicit fear-induced stereotypic behaviors, change activity patterns and feeding rate, and affect the neuroendocrine system, reproductive behavior, and reproductive output in potential prey. Domestic cat is the most specialized predator to the house mouse. Previously we examined the influence of the species specific compound from the cat urine L-felinine on the behavior, neuroendocrine responses and on the reproductive success in mice. Exposures of mice Mus musculus to L-felinine (0.05%) significantly affected reproductive success as well as caused clear corticosterone response (Voznessenskaya, 2014). Neonatal exposure to odorants may influence the function of the olfactory system of an animal and produce changes in its responses to these odorants later in life. Our earlier studies showed that regardless of the odorant (conspecific or heterospecific urine, androstenone), early exposure to it resulted in an increase in a rodent's sensitivity to that stimulus (Voznessenskaya et al. 1995). Exposure to the odorant during two weeks after the pups opened their eyes appeared to induce the greatest level of sensitization, suggesting a sensitive period to such stimuli (Voznessenskaya et al. 1999). Thus, the specific aim of our current study was to examine whether early olfactory experience of mice with chemosignals of cats during "critical" period for sensitization to odors may modulate behavioral or neuroendocrine responses to the target cues later in adulthood. We used three basic approaches: behavioral, hormonal and immunohistochemical. Olfactory thresholds to cat urine and L-felinine were measured with an automated olfactometer (Knosys, USA). Fecal specific glucocorticoid metabolites and plasma corticosterone were monitored using an ELISA technique. Behavioral patterns were analyzed using an open field paradigm (two different modifications). Exposures of mice to cat chemical cues (urine or L-felinine) during "critical period" significantly lowered the olfactory thresholds ($n=10$, $p<0.05$; $n=10$, $p< 0.01$) which is adaptive for detection of the predator under natural conditions. Immunohistochemical studies showed elevated fos-immunoreactivity in accessory olfactory bulb in response to stimulation with L-felinine ($n=8$, $p< 0.001$) in mice neonatally exposed to the target odor relative to the controls. Neonatal exposures also decreased ($n=22$, $p< 0.01$) patterns of passive-avoidance behavior to cat odors and elevated significantly investigatory activity ($n=22$, $p< 0.01$). At the same time corticosterone response to cat urine/L-Felinine stayed unchanged ($n=10$, $p< 0.01$) indicating the innate nature of the response. Early olfactory experience with cat odors produced dissociation in responses to these odors later in the life at the behavioral level and at the hormonal level.

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P76 - De-orphanization and possible regulation of Manduca sexta pheromone receptors.

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The hawkmoth Manduca sexta is an established model to study insect olfaction. Manduca sexta females attract their mates with the release of a species-specific sex-pheromone blend with bombykal (E,Z)-10,12-hexadcadienal and (E,E,Z)-10,12,14-hexadecatrienal being the two major components. While putative pheromone receptors as well as the conserved ion channel Orco were cloned in the hawkmoth, the bombykal receptor is still not identified. Furthermore, the function of Orco and its possible modification via post-transcriptional modifications or second messengers in different insect species is not fully understood. In three different heterologous expression systems the putative moth pheromone receptor protein MsOr1 was coexpressed with MsOrco and could be activated by bombykal. Preliminary experiments with a stable mimick of the trienal activated MsOr4 in HEK293 cells. MsOrco co-expressed with MsOr1 in Xenopus oocytes elicited dose-dependent inward currents upon bombykal application (10 – 300 µM). MsOrco coexpressed with MsOr4 did not respond to bombykal (30–100 µM), while the bombykal receptor of Bombyx mori BmOr3 coexpressed with MsOrco also responded with bombykal (30–100 µM)-dependent inward currents. In addition, HEK293 cells and CHO cells expressing these receptors showed a rise in the intracellular free Ca2+ concentration when stimulated with bombykal. Both proteins, MsOr1 and MsOrco possess putative phosphorylation sites for protein kinase C (PKC). Thus, we tested the effect of inhibition of phospholipase C (PLC) using U73122, or inhibition of PKC using Gö6976 in heterologous expression systems. In both cases the bombykal-responses were significantly reduced. This indicates that PLC and PKC activity appear to be involved in Ca2+ signaling mediated by the bombykal receptors expressed in HEK293 and CHO cells.

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P77 - Functional characterization of human olfactory receptors responding to pyrazine odorants.

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ChemCom progresses towards its objective of deorphanizing the whole repertoire of human olfactory receptors (hORs). Relying on (i) its proprietary technology, (ii) libraries of thousands of odorant compounds and (iii) an efficient screening system, ChemCom is currently identifying and characterizing new modulating molecules (enhancers or blockers) and novel odorant compounds for the whole range of hORs. At ChemCom, more than 120 hORs have been robustly deorphanized. Here, we present a comparative

structure-activity relationship (SAR) data for two unrelated hORs, OR5K1 and OR2AG1, highly expressed in the whole olfactory mucosa. Their most potent agonists were found among pyrazines, with an EC50 below the micromolar range. The SAR study revealed that OR5K1 presents a wider selectivity than OR2AG1. The most potent agonists identified so far have a main olfactory note generally quoted as "nutty" or "green" and are key compounds of several malodors. Linking this SAR study to organoleptic properties suggests that the "nutty" note is associated with OR5K1 only, whereas the "green" note is related to both OR5K1 and OR2AG1. As exemplified by this study, the identification of the combination of activated ORs underlying the perception of a given class of compounds associated with an accurate knowledge of the odorant-olfactory receptor molecular interactions will allow ChemCom to: • perform structural modifications of agonists for reducing or enhancing their notes; • select (the) receptor(s) implied in the perception of a particular odor in order to speed-up the screening for antagonists or enhancers identification (e.g. antagonists of these pyrazines receptors).

P78 - Generation of a cellular sweet taste receptor assay using genomic engineering.

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Sweet taste has an undeniable sensory appeal directing humans toward foods that provide energy and essential nutrients. Consequently, added sugar is a very important ingredient in processed foods fulfilling consumer's demands in taste and enjoyment. However, added sugar gets more and more under scrutiny as driver for obesity and as primary cause for diseases such as heart diseases and diabetes. An ideal solution to reduce caloric intake would be a compound, preferentially from a natural source, which enhances the sweet perception of sugar, allowing for natural sugar taste enjoyment at much lower caloric intake. Sweet tastants are detected by an heteromeric receptor complex formed of the two family C GPCRs TAS1R2 and TAS1R3. In the past decade, several groups approached this receptor by overexpressing genes coding for TAS1R2 and TAS1R3 in mammalian cells followed by subsequent screening campaigns to identify novel sweet taste receptor interacting molecules. Here, we present a new sweet receptor screening assay using advanced genomic engineering technologies based on the CRISPR-Cas9 system. We used the CRISPR-Cas9 technology to modify HEK293T cells and precisely targeted the genomic regions upstream of the transcription start sites of TAS1R2 and TAS1R3. Insertion of a strong constitutive promotor 5' of each gene resulted in the formation of functional human sweet taste receptor units. As a consequence, in this system the human sweet taste receptor is endogenously expressed in its unmodified, original genomic configuration. The expressed receptor is highly sensitive to stimulation with various sweet tastants and the human sweet taste inhibitor lactisole. The incorporation of both fluorescent and luminescent read-out technologies makes this system a versatile tool to investigate agonistic and enhancing activity of a large number of individual natural or artificial compounds, compound fractions and crude natural extracts on the human sweet taste receptor.

P79 - An extraoral taste circuit integrates nutritional state with sensory information for persistent sugar ingestion in Drosophila.

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To survive and adapt to environmental changes, animals need to optimize the amount and quality of food consumed. Food intake is regulated in the nervous system by evaluating chemosensory information and nutritional state of the animal. Taste cells in oral and extraoral sensory organs transmit sensory information to higher order neural circuits that mediate food intake decisions. Here we investigated the function of an extraoral taste circuit in the fly Drosophila melanogaster, and identify sugar sensitive pharyngeal taste neurons that communicate food quality to a compact neural circuit, which integrates nutritional status and taste information to drive persistent food intake. We developed an automated real-time food intake assay, Expresso, which measures individual meal-bouts with nanoliter resolution, and use this system to show that flies regulate their food intake based on their nutritional state and the quality of the food presented. Using intersectional genetics, neural silencing and optogenetic activation, we identify ~10 local interneurons in the primary taste centre of the fly brain that are necessary and sufficient for food intake. Double-labelling experiments showed that extraoral taste neuron terminals overlap with IN1 interneuron arbours in the anterior subesophageal zone. We used the GFP reconstitution across synaptic partners (GRASP) method to show anatomical connectivity between IN1 neurons and the pharyngeal sensory neurons. Our work provides functional evidence for the existence of an extraoral taste circuit that integrates the nutritional status of the fly with taste sensory information to drive persistent sugar ingestion. These findings raise the possibility that extraoral food detection may provide a fast feedback mechanism to assess food quality and optimize food intake based on the internal state of an animal.

P80 - Olfaction and biophilia: A first whiff.

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The term and concept of biophilia (Wilson, 1984; Kellert & Wilson, 1993) characterizes human affective, cognitive, physical and experiential connectedness with the natural environment. Biophilia was turned into a trait of people's environmental

attitudes and the individual level of biophilia shown to correlate with the declarative attention to various features in the environment (e.g., Nisbet et al. 2008). The present study aimed to emphasize a role for olfaction in biophilia in comparing people who evince high vs. low biophilia in terms of: (i) declarative awareness of odors in everyday and natural settings; (ii) ability to identify a series odorants. Sixty participants (32 women, 28 men; mean age: 36 y; range: 19–59 years) completed 3 questionnaires: the nature relatedness scale (NRS, Nisbet et al. 2008) evaluating their environmental concern and behavior, the odor awareness scale (OAS, Smeets et al. 2008) assessing their olfactory responsiveness in everyday situations, and the olfactory awareness in Nature scale (OANS, unpublished) assessing their responses to odour objects/scenes in various natural/urban settings. The participants also performed two identification tasks of 10 familiar odorants [animals (fish and wet dog), flowers (rose and lavender), fruit (apple and banana), trees (pine and burned wood), undergrowth (mushrooms and grass)] following free (uncued) and cued identification procedures (cues provided by experimenters as one correct label among four). The group of participants was divided following a 50th percentile split into high (HB) and low biophilia (LB) subgroups (both men and women equally represented) according to their NRS scores. One-way ANOVAs were performed on OAS and OANS scores and on the number of correct responses in the free and cued identification tasks for the Biophilia (HB vs. LB) and Gender factors. HB participants exhibited higher declarative awareness of odours than LB participants in everyday settings (OAS: $M \pm SD = 121.3 \pm 12.9$ vs. 110.50 ± 16.5; $p = 0.006$), as well as for odours in nature (OANS: $M = 62.77 \pm 7.9$ vs. 53.48 ± 7.5; $p = 0.0001$). But, with the present set of familiar odorants, both subgroups appeared homogeneous in terms of free and cued identification performance. Women declared higher awareness than men, but only towards everyday odours (OAS: $M = 120.9 \pm 2.6$ vs. $M = 110 \pm 2.8$; $p = 0.008$). In conclusion, self-assessed biophilia appears to be related with attention and awareness of odours in the environment, and in particular in the natural and outdoor environment.

P81 - Electrical stimulation of the human olfactory mucosa fails to generate olfactory perception yet alters activity in primary olfactory cortex: a novel path to human brain stimulation.

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The mammalian olfactory system is in contact with the external environment through bipolar sensory neurons with nerve endings in the nasal mucosa. Efforts to artificially stimulate these nerve endings using electrical currents applied in the human nose have yielded mixed results. Here we used endoscopically guided electrode placement to electrically stimulate these targets in 46 healthy human subjects who participated in 139 stimulation sessions. Despite application of varied electrical stimulation parameters, we never once succeeded to artificially induce the perception of odor. In turn, stimulation drove an assortment of non-olfactory sensations, including nasal itching, tingling, cooling, and visual flashes of light (phosphines). Furthermore, sub-detection threshold electrical stimulation applied concurrently with odor drove a small but significant reduction in the perceptual pleasantness associated with the odor ($T(15) = 3.3$, $p < 0.005$). Finally, we tested whether such stimulation is reflected in patterns of brain activation as measured with functional magnetic resonance imaging. We tested 20 subjects, each scanned twice in a 3-Tesla Siemens MRI magnet, once after electrical stimulation and once after sham stimulation. Subjects could not tell which experiment was sham and which was electrical stimulation (Wilcoxon signed rank test $Z = 26$, $p = 0.33$). Nevertheless, sub-detection-threshold unilateral electrical stimulation of the nasal mucosa drove decorrelation of neural activity between left and right primary olfactory cortex ($F(1,18) = 15$ $p = 0.001$). Taken together, we conclude that electrical stimulation of the nasal mucosa fails to generate olfactory perception despite downstream modulation of neural activity. This failure to induce olfactory perception has implications for the interpretation of artificial stimulation studies in olfaction. Although those studies may be highly informative, even if accompanied by selective activation of downstream olfactory targets, one must take caution in assuming that this necessarily implies induction of odor perception. Furthermore, the results of selectively altered brain activity may imply the potential for a novel minimally invasive path to deep human brain stimulation.