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ABSTRACTS

XXVIII[th] Annual Meeting of the European Chemoreception Research Organization, ECRO 2018

Maritim Hotel, Würzburg, Germany, 5–9 September, 2018 Organizers: Wolfgang Meyerhof and Frank Zufall

KEYNOTE LECTURES

Key-01

Olfactory Neuroethology in Flies and Moths

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During ten years of work we have dissected the olfactory system in drosophilia flies and in the sphingid moth Manduca sexta. I will make an excursion to the examples of ecologically labelled lines through the olfactory systems of these insects. From the well-known examples of sex pheromone communication via lines for food-and oviposition-site-search to lines mediating avoidance of enemies and harmful microbes. All of these lines have the characteristic that the resulting behaviour after activation of the line can be predicted with high certainty.

Having ecologically labelled lines in a sensory system obviously has its advantages but it also opens up for exploitation by other organisms. In this context we have studied both flowers and microbes that manipulate insects by activating olfactory sensory neurons being the detecting part of neural circuits mediating attraction either for sexual or egg laying behaviour.

Presently, we are applying genome editing to dissect the moth olfactory sense more in detail. As these experiments are presently ongoing I will report on new results.

Key-02

Toward neuroendocrine precision medicines for metabolic diseases

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Emerging insights from recent advances in metabolic diseases research suggest that one or several patterns of multiple neuroendocrine factors are necessary for sustained modulation of body fat or metabolism set points. Gut hormones appear to reside at the core of these master–key-like signaling patterns, as indicated for example by bariatric surgery research. Over the last 10 years, we have therefore tested several series of unimolecular combination therapeutics candidates based on multiple gastrointestinal and adipocyte derived signals. Balanced single molecule peptide hormone based GLP1-glucagon and GIP-GLP1 co-agonists exhibited superior body weight loss and glucose metabolism benefits in mouse models of obesity and diabetes, as compared to any established mono-agonists. Preliminary translational data indicate efficacy of GIP-GLP1 co-agonists in non-human primates. Since coinfusion of a soluble and stable glucagon mono-agonist in parallel with GIP-GLP1 coagonist treatment provided additional benefits, a series of single molecule GIP-GLP1-glucagon triagonists were generated and validated. These novel triagonists again showed unprecedented metabolic and body weight benefits in mouse and rat models of obesity and diabetes even beyond dual agonism. In a parallel approach single molecule conjugates combining a peptide (GLP1, glucagon) with a steroid (estrogen, thyroid hormone, glucocorticoids) were generated to maximize metabolic benefits and minimize potential toxicity by specifically targeting a subset of nuclear receptors in peptide-receptor carrying cells. Such peptide carrier based targeting of a specific subset of nuclear hormone receptors was successful: Administration reversed hallmarks of the metabolic syndrome in diet induced obese and insulin resistant mice without causing any detectably side effects or toxicity. Several of these novel single molecule approaches to polypharmaceutical therapeutics are now advancing up to phase 3 clinical studies.

Key-03

Reflections on the revolutions in olfaction

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We became acquainted in the 1970s, one of us (GS) working in the field of mammalian olfaction, the other (JH) a transplant

from a different field working on insect olfaction. Through our interactions we each learned about the work of others. GS was introduced to Juergen and Vera Boeckh, Karl-Ernst Kaissling, and others of the insect community, and learned a new vocabulary that seemed strange to a mammalian physiologist! JH met Al Farbman, Charles Greer, John Kauer, Doron Lancet, and others advancing understanding of olfaction in vertebrates. We figured out that we were scientifically united by our interest in the glomerulus, and from there we've enjoyed being part of the effort over more than forty years leading from one revolution to another in understanding the common principles underlying olfaction across species, and the adaptations that make species special. We'll review some of those revolutions and the role that ECRO played in promoting the science, and reflect on so many rewarding friendships and collaborations along the way.

Key-04

Revealing diverse vomeronasal ligands affecting behavior and emotion in mice

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The mouse vomeronasal organ located between nasal and oral cavities detects a variety of chemical signals in the external environment. For example, a male-specific lacrimal protein ESP1 activates a specific vomeronasal receptor V2Rp5 and enhances sexual receptive behavior in female mice (Haga et al. Nature 2010) and aggression in male mice (Hattori et al. Current Biol. 2016). ESP1 is also involved in the Bruce effect in pregnant females (Hattori et al. Current Biol. 2017), suggesting the presence of sexual dimorphic and context-dependent signaling pathways. We recently found that a juvenile pheromone ESP22 not only suppressed sexual mounting behavior in adult males (Ferrero et al. Nature 2013) but also enhanced sexual rejection in females via V2Rp4 (Osakada et al. submitted). It appears that different pheromones (ESP1 or ESP22) and, thus, different vomeronasal receptors (V2Rp5 or V2Rp4), couple with distinct limbic neural circuits, leading to opposing effects on females. We dissected the specific amygdala-hypothalamus-midbrain pathways leading to the ESP1 or ESP22-mediated behavior (Ishii et al. Neuron 2017). The VNO senses not only pheromones but other signals including kairomone. We identified a novel vomeronasal ligand, cystatin-related protein 1 (CRP1), from male rat tear fluid that mediates an intra-specific signal in a predator species (rat) and also function as an inter-specific kairomone signal in the prey species (mouse) through specific vomeronasal receptors including Vmn2r28 (Tsunoda et al. Current Biol. 2018). Supported by ERATO Touhara Chemosensory Signal Project, JST, Japan.

Key-05

Smell and stress in mammals

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Mammals detect a vast array of chemicals in the external environment. These include a multitude of chemicals perceived as odors as well as pheromones and predator odors, which stimulate hormonal changes or instinctive behaviors. We have explored how the olfactory system detects diverse odorants and how the nervous system translates odorants into diverse perceptions and innate responses. We identified four different families of chemosensory receptors expressed by sensory neurons in the mouse olfactory epithelium or vomeronasal organ. The largest is the odorant receptor (OR) family, which mediates odorant detection in the nose. Our studies showed that ORs are used in a combinatorial fashion to encode odor identities, that individual olfactory sensory neurons express a single OR gene each, and that information provided by 1000 different ORs is highly distributed in the nose but transformed into a semi-stereotyped map of OR inputs in the olfactory bulb. In recent studies, we investigated how predator odors induce innate physiological fear responses. Using viral tracers and chemogenetic techniques, we found that one small brain area comprising less than 5% of the olfactory cortex plays a major role in inducing increases in stress hormones in response to volatile predator odors detected in the nose. Our studies also revealed a complex pattern of neural pathways that convey signals about different stressors to hypothalamic CRH (corticotropin releasing hormone) neurons, which control stress hormone levels. We are currently working to generate a molecular map of neural identities to superimpose on the anatomical map, laying a foundation for future studies to dissect the roles of different neurons and chemical messengers in the control of stress.

SYMPOSIUM 1: IONOTROPIC SENSORY RECEPTORS: USING CHANNELS TO DETECT THE CHEMICAL WORLD

S1

A hot TRP channel on steroids

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In the endings of sensory nerves in the skin and mucosa of mammals, thermal and chemical cues are translated into neuronal activity to initiate somatosensory processes including pain. We investigated the molecular sensors involved in the detection of noxious heat, and revealed a

central role for the transient receptor potential (TRP) ion channel TRPM3. We found that TRPM3 is expressed in nerve endings of C and Aδ fibers in the skin, where it responds both to noxious heat and to specific neurosteroids. TRPM3 is absolutely required for the pain response to neurosteroids, and is one of three redundant TRP channels that mediate the acute pain response to noxious heat. Inhibition of TRPM3 expression or function has strong analgesic effects in various rodent models of acute and chronic pain, suggesting that TRPM3 may be an interesting new target for analgesic drug development.

S2

Ionotropic receptors as multi-modal sensory receptors

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Since their identification as a novel branch of the Ionotropic Glutamate Receptor family (a family that includes NMDA, Kainate, AMPA Receptors), the Ionotropic Receptors (IRs) have emerged as a major family of invertebrate sensory receptors (Benton et al., 2009). While primarily characterized as a large receptor family whose members mediate the detection of diverse chemicals, recent studies reveal that some of the most evolutionarily conserved IRs mediate temperature and humidity detection in Drosophila (Enjin et al., 2016, Frank et al., 2017; Ni et al., 2016, Knecht et al., 2016, Knecht et al., 2017). IR-dependent chemosensing commonly depends on multiple IRs, with shared "co-receptor" IRs working with various "modality-specific" IRs to confer different chemical specificities. IR-dependent thermo- and hygro-sensing appears to operate similarly: IR25a and IR93a act together with IR21a, IR40a and IR68a to mediate the detection of cooling, dry air and moist air, respectively. We are now exploring the mechanisms by which these receptors mediate thermo- and hygro-sensory detection in insects using a combination of genetics, physiology, ultrastructure and behavior. These findings indicate that Ionotropic Receptors are responsible for mediating critical temperature and humidity detection transduction events previously attributed to TRP channels. By probing the physiological responses and behavioral roles of these IR-dependent sensory neurons, we find that IR-dependent phasic sensory detection is essential for mediating behavioral responses to innocuous sensory cues. Finally, at the molecular level, IRs are found to not only mediate transduction, but to be capable of organizing a neuron's sensory compartment by controlling the elaboration of highly structured membrane:membrane contacts. Together these studies begin to address the role of IRs in sensory encoding and behavioral control and reveal a previously unexpected role for IRs in neuronal morphogenesis.

S3

Ionotropic receptors: a family of mineral taste receptors

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In many animals, the taste of sugars is attractive and the perception of bitter is aversive. However, the taste of minerals such as Na^+, Ca^{2+} and H^+ is more complicated. Low Na^+ is appealing, while high Na^+ is repulsive. The taste of H^+ is also bivalent. Ca^{2+} is an essential mineral. Yet, high levels are toxic. To dissect the mechanisms of mineral taste, we are focusing on Drosophila. We found that the decision to feed or reject foods with Na^+ depends on competition between gustatory receptor neurons (GRNs) activated by low versus high Na^+. Moreover, we found that a Na^+-permeable Ionotropic Receptor (IR76b) is the low Na^+ receptor. Surprisingly, IR76b is a gateless channel. Activation of the low salt GRNs depends on an increase in extracellular Na^+ by exposure to Na^+-containing food, and Na^+ influx via IR76b.

The taste of Ca^{2+} is especially enigmatic, as it is unclear if many animals such as flies are endowed with this taste. We found that high Ca^{2+} is aversive, and the suppression is mediated by of a specific class of Ca^{2+} activated GRNs that requires several members of the IR family (IR25a, IR62a, and IR76b). Consistent with Ca^{2+} rejection, high levels of Ca^{2+} decrease survival. Thus, detection of Ca^{2+} represents an additional taste in Drosophila, and is required for avoiding toxic levels of this mineral.

The receptors that detect sour compounds and influence feeding are elusive, although in mammals Otop1 is a prime candidate. We revealed that an Ionotropic Receptor (IR7a) is essential in flies for rejecting foods laced with aversive levels of acetic acid. IR7a was dispensable for repulsion of other acidic compounds, indicating that acid taste occurs through a repertoire rather than a single receptor. IR7a was expressed in a subset of bitter GRNs, rather than GRNs dedicated to sour taste. Our findings suggest that flies taste acids through multiple receptors, enabling them to discriminate foods on the basis of acid composition, rather than just pH.

S4

Otopetrins: Proton channels in sour taste cells and beyond

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Of the five basic tastes, sour, which allows animals to sense acids present in citrus and spoiled foods, remains one of the least understood. Sour taste is transduced by a subset of taste cells that lie within taste buds in the tongue and palate epithelium and express a number of cell-type specific markers, including the Pkd2l1 gene [8, 12–14]. Until recently, there was little information on the ionic conductances that underlie the electrical responses of murine taste cells to sour, limiting our understanding of this fundamental sense. By patch clamp recording from genetically identified PKD2L1-expressing taste cells, we have shown that in PKD2L1 cells, but not in other taste cells, strong acids elicit an inward current carried entirely by protons (H+). To identify the gene that encodes the proton channel, we performed differential transcriptome analysis of PKD2L1 and TRPM5-expressing taste cells and tested candidates in heterologous expression systems. One gene, Otop1, encoding a twelve transmembrane protein, generated large currents in response to acidic stimuli in both Xenopus oocytes and HEK-293 cells, like those found in taste cells. We also provide evidence that Otop1 is required for proton currents in taste cells, taking advantage of a naturally occurring mutation in Otop1 that interferes with trafficking of the channel. Otop1 is a member of an evolutionarily conserved gene family, many of which also encode proton channels. The function of this new family of ion channels, which are widely distributed throughout the body, remains largely to be determined.

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SYMPOSIUM 2: RECENT DEVELOP-MENTS IN STATE-DEPENDENT MODULATION OF THE EARLY RODENT OLFACTORY SYSTEM

S5

Prioritizing olfaction

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Odors guide complex multisensory behaviors in remarkable manners, indicating that brain systems exist which allow for the prioritization of olfactory information in the presence of competing extramodal stimuli. Indeed, higher order cognitive processes, such as navigation, learning and memory, and action selection, rely on the proper filtering of sensory cues based on their salience. We asked what effects selective attention has on rodent olfactory behavior and neural system function? To address this, we developed a quantitative reward-guided behavioral paradigm named the Carlson Attention Task (CAT). Through the CAT, we harnessed the selective attention of rats to odors and revealed that selective attention facilitates accurate odor-guided decisions, which become further strengthened with experience. Further, we uncovered that selective attention to odors adaptively sharpens their representation among neurons in the olfactory tubercle, an olfactory cortex region we have uncovered is involved in evaluating sensory information in the context of motivated behaviors. Odor-directed selective attention exerts influences during moments of heightened odor anticipation and enhances odorant representation by increasing stimulus contrast in a signal-to-noise type neural coding scheme. Together, these results reveal that rats engage selective attention to optimize olfactory outcomes. We have also begun to test if and how the frontal cortex orchestrates the influence of attention on olfaction. Overall, this work is yielding new information regarding how our cognitive state allows for us to place priority on odors in adaptive manners.

S6

Cortical top down control of early olfactory coding and social recognition

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Oxytocin promotes social interactions and recognition of conspecifics that rely on olfaction in most species. The circuit mechanisms are incompletely understood through which oxytocin modifies olfactory processing. We observed that optogenetically induced oxytocin release enhanced olfactory exploration and same-sex recognition of adult rats. Consistent with oxytocin's function in the anterior olfactory cortex particularly in social cue processing, region-selective receptor deletion impaired social recognition, but left odor discrimination and recognition intact outside a social context. Oxytocin transiently increased the drive of the anterior olfactory cortex projecting to olfactory bulb interneurons. Cortical top-down recruitment of interneurons dynamically enhanced the inhibitory input to olfactory bulb projection neurons and increased the signal-to-noise of their output. Thus, oxytocin generates states for optimized information extraction in an early cortical top-down network. Yet, the top-down and bottom up interactions between the olfactory bulb and anterior olfactory cortex are largely unknown. We therefore examined with multi-site parallel multiple single unit recordings in awake mice the interaction of the two brain structures with

particular consideration of attentional modulation and presentation of pure odors and odor mixtures. In summary, oxytocin generates states for optimized information extraction in an early cortical top-down network that is required for social interactions with potential implications for sensory processing deficits in autism spectrum disorders.

S7

CUBIC-HistoVIsion: a pipeline for three-dimensional whole-brain/organ staining and imaging with single-cell resolution

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Recent development of various tissue clearing and three-dimensional (3D) imaging methods enabled the comprehensive observation of whole organ/body with cellular resolution or more. Several studies tried to integrate whole-mount staining into the clearing-imaging scheme. However, due to the difficulty in efficient penetration of stains and antibodies, they have only been applied in loose embryonic tissues or with a limited number of antibodies/stains for adult rodent tissues. To logically find out critical parameters for the efficient penetration, we first investigated chemical features of fixed and de-lipidized tissue as a type of gel. Then, we constructed a surrogate assay with an artificial gel similar to tissue gel, in order to widely examine multiple chemical conditions for efficient staining. The identified parameters were integrated as a general 3D staining protocol, with which we have confirmed ~30 chemicals and antibodies used in whole adult mouse brain staining and imaging with single-cell resolution. The developed "CUBIC-HistoVIsion" pipeline for 3D histology and volumetric imaging provides opportunities for multi-channel imaging of functional and structural molecules of whole adult mouse brains as well as a primate brain thus will be widely applied to neuroscience researches in future.

S8

Top-down inputs to the mouse olfactory bulb

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The olfactory bulb (OB) receives top-down input from two major types of modulatory systems whose role in shaping early olfactory processing remains unclear: classical neuromodulatory inputs and cortical inputs.

We found that neuromodulatory cholinergic projections from the horizontal band of broca (HDB) regulate OB output by increasing the spiking frequency of mitral/tufted (MT) cells, the principal OB output neurons. In contrast, GABAergic HDB projections strongly reduce the spiking frequency of MT cells. These modulations were rapid and transient. HDB modulation of MT cell spiking occurred independent of sensory input strength, indicating robust and differential modulation capacities of cholinergic vs GABAergic HDB projections.

Additionally we investigated projections from the anterior olfactory nucleus (AON) a major source of cortical feedback to the OB. Stimulating AON projections also robustly regulated OB output by decreasing the spiking frequency of MT cells. Similar to HDB effects, this modulation was rapid and transient. The AON has been shown to send sensory-evoked feedback to the OB. However, AON modulation of MT cell spiking was nearly independent of sensory input strength. So far, silencing AON projections revealed only mild effects on MT cell activity.

These experiments demonstrate that: 1) A differential modulation of neuronal responses can not only be achieved by activating different top-downs system but can also be mediated via different neuronal populations of the same top-down area. 2) Despite recent reports pointing to a potential coexpression of cholinergic and GABAergic markers, our data argue for a functionally distinct neuronal population. 3) The interplay between different top-down systems might be important for dynamically regulating the sensitivity to or salience of odors during active sensing 4) These modulations are likely to represent local effects mediated by neurotransmitter release at the level of the OB.

SYMPOSIUM 3: WHEN SENSES TAKE FLIGHT: THE CHEMICAL SENSES OF BIRDS

S9

Behavioural and neuro-ethological evidence for olfactory chick recognition by male zebra finches

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For a long time it was assumed that songbirds do not possess a functioning sense of smell. Likewise, olfactory kin recognition was only scarcely investigated. Especially, during parental care, male songbirds were often assumed to base their feeding investment solely on spatial cues, ignoring phenotypic cues of the offspring.

Recent studies already proofed the use of the sense of smell in social contexts like conspecific or kin recognition in a variety of avian species. In the present experiment we use head saccades as a measure for the arousal of male zebra finches and confirm that zebra finch males differentiate between familiar, related and foreign offspring based on odour cues.

Additionally we tackle the question how such social stimuli are processed in the avian brain. By using the expression of the immediate early gene product c-Fos as an activity marker, we show that the activation pattern, i.e. the activity difference between the left and the right hemisphere, of several hippocampal areas is altered by the presentation of the own nestlings' odour in comparison to a control odour. The hippocampus is therefore likely involved in odour based social recognition.

Taken together our experiments provide behavioural and neuro-ethological evidence for the existence of phenotypic cues of nestlings, i.e. body odours, and for their perception by male songbirds.

S10

Odor-based mate choice in birds.

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Many animals are known to preferentially mate with partners that are dissimilar at the major histocompatibility complex (MHC) in order to maximize the disease resistance of their offspring. Numerous taxa, including fish, lizards and mammals, are known to use odors to assess MHC similarity with potential partners. In contrast, the ability of birds to assess MHC similarity using olfactory cues has not yet been explored. Here we studied two seabirds, the black-legged kittiwake and the blue petrel, that are known to preferentially mates with genetically dissimilar partners, and tested whether these birds can discriminate the odor of conspecifics that vary in their MHC similarity. First, we found that similarity in preen secretion chemicals was positively correlated with MHC relatedness in male-male and male-female dyads in kittiwakes. Second, using behavioral discrimination tests in kittiwakes and petrels, we found that these species can estimate MHC similarity based on odor. These results provided the first evidence that odors encode information on MHC relatedness in birds and suggest that MHC odor cues might be vertebrate-wide

S11

Molecular features underlying selectivity in chicken bitter taste receptors

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Chickens sense the bitter taste of structurally different molecules with merely three bitter taste receptors (Gallus gallus taste 2 receptors, ggTas2r1, ggTas2r2 and ggTas2r7), representing a minimal case of bitter perception. Some bitter compounds activate all three ggTas2rs, while others selectively activate one or two of the receptors [1].

The combination of computational approaches with site-directed mutagenesis was successfully used to characterize the agonist-bound conformation of the bitter taste receptor binding sites. We first investigated the binding modes of several known agonists in ggTas2r1 and successfully used the ggTas2r1 structural model to identify three quinine analogs as new ggTas2r1 agonists [2]. Next, we characterized the selectivity profile of all ligands against all three ggTas2rs and identified the residues responsible for receptor selectivity [3].

ggTas2r modeling proves to be a valuable interpretative tool to shed light on the molecular recognition complexity of the bitter taste in chicken. Interestingly, promiscuous compounds are predicted to establish polar interactions with position 6.51 and hydrophobic interactions with positions 3.32 and 5.42 in all ggTas2rs; whereas specific residues are responsible for receptor selectivity. Therefore, we used the constructed models to virtually screen BitterDB compounds (bitterdb.agri.huji.ac.il). ~50% of compounds known to be bitter to human are likely to be bitter to chicken, with 25%, 20%, 37% predicted to be ggTas2r1, ggTas2r2, ggTas2r7 agonists, respectively. Predicted ggTas2rs agonists could be valuable for in-vitro and in-vivo experiments, contributing to the improvement of chicken feed and to our understanding about the bitter taste.

1. Di Pizio, A. and M.Y. Niv, Bioorgan Med Chem 2015. 23:4082.
2. Di Pizio, A., et al., Sci Rep 2017. 7:8223.
3. Di Pizio, A., et al., Front Mol Biosci 2018. 5:6.

S12

How do birds cope with chemically defended foods?

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Noxiousness, unpalatability, and toxicity are some of the defences that plants and animals have evolved to avoid being eaten. In many cases these chemical defences are signaled to visually hunting predators, like birds, via conspicuous warning colours. Birds learn to associate the visual signal with the taste, flavor, or negative effects of ingesting the chemical defence, and subsequently reduce their intake of

prey with similar visual signals. However, bird species vary in their sensitivity to chemical defences. Some birds have evolved resistance to prey toxins; others have adapted behaviors to overcome chemical defences. I will present research investigating within species variation in dealing with unpalatability, and research on between species resistance to toxicity. Both within- and among-species differences in the handling of distasteful or toxic prey can provide novel insights into chemosensory perception in non-model organisms

SYMPOSIUM 4: OLFACTION AS A MODERATOR OF HUMAN RELATIONSHIPS

S13

Chemosensory danger detection in the human brain

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For detection of dangers, the sense of smell has proven an important role. Besides environmental dangers, also socially dangerous situations are commonly communicated via odors in mammals within a species: For humans, first evidence of the communication of aggression and competition-related information via body odor is arising. We here ask the following questions: 1. Is an intention to attack conveyed via body odor from a male combative sender to a recipient? 2. Do aggression chemosignals activate a neural alarm system in the brain? To answer those questions, body odors during an exercise and an aggression condition were sampled from 16 healthy male donors and were used during a behavioral and a neuroimaging application study both using an emotional Stroop paradigm. The behavioral data indicate an exclusive impairment of the processing of anxiety-related words by aggression chemosignals which is interpreted as time-sensitive attentional bias in chemosensory danger detection. During exposure to aggression chemosignals compared to exercise chemosignals, functional imaging data exhibit an activation of thalamus, hypothalamus and insula. Together with the thalamus, the ACC was seen activated in response to threat-related words only. Impact and limitations of aggression chemosignalling on human behavior will be discussed.

This research was supported by a grant from the Interdisciplinary Centre for Clinical Research within the faculty of Medicine at the RWTH Aachen University, Germany.

S14

Social chemosignals in autism spectrum disorder: a tale of neurodiversity

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The characterization of olfactory skills in autism spectrum disorder (ASD) has recently spurred the interest of scientific research. Besides anecdotes suggesting that individuals with ASD exhibit abnormal chemosensory experiences, now behavioral and psychophysiological data confirm this phenomenon. However, the behavioral manifestations and neural mechanisms of mature common and body odor perception in ASD are still unclear. Here, we investigate odor identification skills and the structural and functional underpinnings of olfactory perception in 33 adults with ASD (13F) and 39 controls (19F). Results indicate that: i) ASD are ~3 times more likely to show reduced odor identification skills than controls; ii) odor identification skills are underlined by significant changes in the tractography of the frontal part of the inferior fronto-occipital and the inferior longitudinal fasciculus across groups; iii) ASD and TD processed body odors in distinct networks from the nonsocial common odor. However, the network used are not overlapping. Behavioral results are discussed in the context of piriform cortex, orbitofrontal, and limbic functional activations as well as ASD severity. Taken together, our findings describe for the first time explicit and implicit olfactory behaviors and their neural underpinnings in adults with ASD and suggest that olfactory perception and neuroimaging can provide non-invasive tools for ASD characterization.

S15

Effect of hormonal contraception use on preferences for MHC-heterozygosity

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Preferences for body odour of genetically heterozygous individuals were reported in various vertebrate species. However, the evidence in humans is inconclusive perhaps because most previous studies did not control for use of hormonal contraception (HC). To test this question, we collected body odour samples from 52 men who were genotyped in their MHC A, B, DR loci. The body odour samples were subsequently rated for attractiveness by a panel of HC users (N = 23) and HC non-users (N = 29). Overall, there was no significant difference in attractiveness of body odour between homozygous and heterozygous individuals. Interestingly, women using HC tended to prefer homozygous individuals. Our

results indicate that preferences for heterozygosity can be modulated by steroid hormones.

S16

Body odor disgust scale (BODS): Its validation and association with social biases.

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Body odors provide important social and health-related cues in many species. While human body odor perception often triggers feelings of disgust, few studies have investigated body odor disgust in a systematic way. We have developed the body odor disgust scale (BODS), a brief 12-item scale to assess the extent to which individuals are disgusted by common body odors such as sweat and urine. The scale development included both internal and external validation tests. We used the BODS in conjunction with scales measuring social attitudes and biases, and found consistent associations between high body odor disgust and stronger authoritarian attitudes, as well as more pronounced outgroup biases. Our work is consistent with the "behavioral immune system" framework, wherein social attitudes and political ideologies are shaped by perceived pathogen risk and disease avoidance via feelings of disgust. Body odor perception may thus not only be important for personal interactions, but may also be linked to social attitudes and political ideologies.

S17

Olfaction as a moderator of parent-child bonding: New insights on the base of a HLA genotyped family cohort

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Body odors play an important role for kin recognition, which facilitates bonding to family members and may prevent from inbreeding. The smell of a baby´s body odor is related to central reward processing and is assumed to enhance caregiving behavior in mothers. Therefore, exposure to body odors may serve as an approach for interventions targeting parent-child relationships. Environmental (e.g. familiarity), developmental (hormones) and genetic compounds (e. g. human-leucocyte antigen, HLA) are assumed to interplay in body odor perception. We systematically investigated the parental perception of children's´ body odors (aged between 0 and 18). We hypothesized that the interaction of age, familiarity and HLA-similarity affects parental ratings. The sample up to now consisted of 158 mothers (M = 37.5, SD = 7.8 years); 42 fathers (M = 37.5, SD = 8.4 years); M = 1.8 children per family (SD = 0.7); 199 children; 108 girls (M = 7.6, SD = 5.9 years). Participants completed questionnaires regarding parent-child relationship. Parents had to assess intensity, wanting, sweetness and attraction of six body odor samples, as well as to identify the own children´s body odor. HLA similarity was defined at least 1 out of 4 alleles similar in HLA-B and –C. Preliminary data revealed that the body odor of the own child is preferred and identified among all age groups. HLA affected pleasantness ratings for the body odors of children younger than three years but did not moderate attraction ratings. Parents were able to identify their children´s body odor correctly and preferred the own body odor across all age groups. Those results suggest that familiarity of body odors plays an important role for parent –child bonding. Further research including all HLA-loci as well as the hormonal status is obtained in order to strengthen the preliminary results and to draw clinical implications.

SYMPOSIUM 5: CHOSEN FROM ABSTRACT SUBMISSIONS

S18

The taste of ribonucleosides: Novel macronutrients essential for larval growth are sensed by Drosophila gustatory receptor proteins

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Animals employ various types of taste receptors to identify and discriminate between different nutritious food chemicals. These macronutrients are thought to fall into three major groups: carbohydrates/sugars, proteins/amino acids and fats. Here, we report that Drosophila larvae exhibit a novel appetitive feeding behavior towards ribose,

ribonucleosides and RNA. Here, we report the identification members of the Gustatory receptor (Gr) subfamily 28 (Gr28), expressed in both external and internal chemosensory neurons, as molecular receptors necessary for cellular and appetitive behavioral responses to ribonucleosides and RNA. Specifically, behavioral preference assays show that larvae are strongly attracted to ribose or RNA containing agarose in a Gr28-dependent manner. Moreover, Ca2+ imaging experiments reveal that Gr28a expressing taste neurons are activated by ribose, RNA and some ribonucleosides, and that these responses can be conveyed to Gr43aGAL4 fructose sensing neurons by expressing single members of the Gr28 gene family. Lastly, we establish a critical role in behavioral fitness for the Gr28 genes by showing that Gr28 mutant larvae exhibit low survival rate when challenged to find ribonucleosides in food. Together, our work identifies a novel taste modality dedicated to the detection of RNA and ribonucleosides, nutrients that are essential for survival during the accelerated growth phase of Drosophila larvae.

S19

Pharmacology of odorant interactions at odorant receptors in vivo

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The interactions between odorants at odorant receptors (ORs) are suspected of playing important roles in olfactory phenomena: mixture suppression and synergism, unpredictable sensory outcomes in formulating fragrances and pairings of foods and drinks, and the perception of complex odors as odor objects versus elemental analysis of the odorant mixture. To identify odorant interactions at ORs, we used an in vivo assay in mice (McClintock et al., 2014, J Neurosci 34:15669) to determine OR response patterns of 4 odorants and test 2 binary mixtures. We investigated mechanisms of interaction using heterologous expression of ORs in cultured cells. Whiskey lactone, which suppresses human perception of isoamyl acetate, also suppresses mouse OR responses to isoamyl acetate in vivo and in vitro. Whiskey lactone is a partial agonist at Olfr213 and Olfr1411. Its weak efficacy causes dose-dependent suppression as it competes with isoamyl acetate at these ORs. Mixtures of bourgeonal and undecanal, which humans perceive as containing both odorants, show more diverse interactions. Both odorants are agonists for Olfr1420, which shows an additive response to mixtures of these odorants in vivo and in vitro. Bourgeonal moderately suppresses undecanal responses of Olfr774 and Olfr1019, where it appears to act as an inverse agonist and antagonist, respectively. In addition, bourgeonal is an inverse agonist of Olfr577, reducing its constitutive activity. Undecanal is a mild antagonist of Olfr16 responses to bourgeonal. Overall, we identified 30 ORs responsive to individual odorants in vivo and confirmed that in vivo odorant interactions at 6 of these ORs could also be measured in vitro. These interactions include examples of additive responses and all possible types of negative pharmacological effects: antagonism, inverse agonism, and competition by partial agonists.

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S20

DNA methylation pattern of an odorant receptor gene

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There are ~1000 mouse OR genes spread throughout the genome. Each olfactory sensory neuron expresses one single OR gene, in a monoallelic fashion. In addition, different members of the OR gene family show unequal levels of gene expression in the olfactory epithelium. The mechanisms underlying OR gene choice and levels of expression are not well understood. Little is known about the role played by DNA methylation in OR gene regulation. Here we analyzed the DNA methylation pattern of the Olfr17 (P2) OR gene in olfactory sensory neurons using the bisulfite conversion method. We found that while the CpGs in the coding region of the gene are frequently methylated, CpGs in the promoter region show lower frequencies of methylation. Interestingly we show that two single nucleotide polymorphisms (SNPs) lead to the occurrence of two additional CpGs in the promoter region of Olfr17 from the 129-mouse strain, which are lacking in the Olfr17 allele from the C57BL/6 strain. As a result, the promoter region of the 129 Olfr17 allele shows higher frequencies of DNA methylation than the one from the C57BL/6 Olfr17 allele. Finally, as revealed by RT-qPCR experiments and in situ hybridization experiments, transgenic mice carrying the 129 Olfr17 allele show reduced expression levels of Olfr17 and reduced number of Olfr17-expressing olfactory neurons of the Olfr17 when compared to transgenic mice carrying the C57BL/6 Olfr17 alleles. Our results indicate that genetic variations in the proximal-promoter regulatory regions can contribute to unequal DNA

methylation frequencies and consequently different expression levels of OR gene alleles.

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S21

Altered social behavior and sensory responses to pheromones in mice deficient for the G-protein subunit Galphai2

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In mice, social behaviors such as mating and aggression are mediated by pheromones and related chemosignals. Olfactory information carried by chemosignals is processed in the accessory olfactory system by sensory neurons from the vomeronasal organ (VNO). Distinct populations of sensory neurons coexist in the VNO, each with distinctive signaling molecules. Subsets of VNO neurons differ in their expression of chemosensory receptors operated by different G-protein subunits [either Gnai2 (Gαi2) or Gnao1 (Gαo)]. The in vivo, physiological roles of each of these segregated neural pathways remain unclear. To address this question, we used conditional gene targeting applying Cre-lox-mediated recombination to create a mouse line carrying a tissue-specific disruption of the Gnai2 gene in all Omp-positive cells. Mutant mice show a normal main olfactory epithelium and do not display major deficits in odor perception. In contrast, the number of VNO neurons that normally express Gnai2 was diminished, with a 30% reduction of cells. We used calcium imaging methods to evaluate the requirement of Gnai2 for the detection of pheromones. Neuronal activation by small organic pheromones was drastically reduced in the Gnai2 mutants indicating that this G-protein is necessary for the detection of these chemosignals. By contrast, detection of peptide and protein ligands specific for Gαo-expressing VSNs was not affected. We also analyzed the mutant mice for different instinctive behaviors. In males, the display of territorial aggression and parenting behavior toward pups was severely altered, whereas other socio-sexual behaviors remained unchanged. These findings indicate that Gnai2 is required for maintenance of the VNO neuronal population and for the detection of pheromones that involve the initiation of social behaviors.

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S22

Neuronal responses in the accessory olfactory bulb following mating in female mice

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The pregnancy block effect (Bruce Effect), remains one of the most striking examples of plasticity in the context of the chemical senses. In this phenomenon, the odors of the mating male are imprinted in the female brain, preventing them from inducing pregnancy block. Several lines of evidence suggest that the pregnancy block is mediated by the vomeronasal system, and that the site of learning is the accessory olfactory bulb (AOB). In particular, it was suggested that protection of pregnancy from the mating male's odors is due to suppression of responses to its odors, a compelling hypothesis known as the "negative template hypothesis". In this study, we set out to test the predictions of this hypothesis by recording real time responses in-vivo in naïve and mated females. According to the hypothesis, neurons in the AOB of mated females should display markedly attenuated responses to odors from the stud males. Our preliminary analyses indicate that this is not the case. Thus, mating does not lead to a global, nor to a consistent suppression of responses to stud male odorants. These results suggest that at least in its simplest formulation, the negative template hypothesis does not account for learning in the context of the Bruce effect. This does not rule out a central role for the AOB in this form of learning, but it does suggest that the physiological fingerprints of learning will be more subtle, expressed either in responses to more complex temporal activation patterns, to changes in population level activity, or to changes confined to a specific subset of neurons.

This reserach is supported by the BSF (United-States Israel Binational Foundation).

S23

Neonatal olfactory memory induces attractive responses even to aversive odorants in adult mice

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During the development of sensory systems, there is a narrow time-window that allows plastic but irreversible changes in neural circuits by environmental inputs. Here we study the olfactory imprinting during this critical period in mice. When the newborn is exposed to a particular odorant, the responding glomeruli become larger and imprinted memory is established. This early exposure induces attractive responses to the imprinted odor and reduces stress reactions even

when the odorant is innately aversive. Activity-dependent Semaphorin 7A expression, which triggers post-synaptic events in the olfactory bulb, is key to imprinting olfactory memory. Blockage of Semaphorin 7A/Plexin C1 signaling in neonates causes impairment of social responses as adults, leading to avoidance of unfamiliar mice. Furthermore, imprinted odor memory suppresses the amygdalo-piriform transition area and newly activates the anterior medial-amygdala, inducing attractive responses. Knockout and rescue experiments demonstrate that oxytocin is responsible for adding the attractive quality to imprinted odor memory. Our present study gives new insights into our understanding of neonatal imprinting during the critical period and neurodevelopmental disorders, such as ASD and attachment disorders.

S24

From local to global signalling in olfactory bulb granule cell dendrites

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The inhibitory axonless olfactory bulb granule cells (GCs) form reciprocal dendrodendritic synapses with mitral and tufted cells, the principal neurons of the olfactory bulb, via large spines. GC dendrites are highly excitable in multiple ways: Synaptic inputs to individual GC spines can generate Na^+ spikes within the spine head, and stronger activation results in globally propagating signals that encompass both low-threshold Ca^{2+} spikes (LTS) and Na^+ spikes.

To investigate dendritic integration we implemented a holographic two-photon uncaging system which allows simultaneous photostimulation of multiple spines in 3D. We uncage glutamate at sets of spines on GC dendrites in acute juvenile rat brain slices while recording the membrane potential from the soma and two-photon Ca^{2+} imaging within one focal plane. Although GC resting potentials are hyperpolarized, we find that the threshold for global GC Na^+ spiking is attained at similar numbers of simultaneously activated spines (9 ± 2) as in cortical pyramidal cells, whereas activation of 5 ± 2 spines suffices to elicit Ca^{2+} signals in dendrites remote from the stimulated spines, possibly corresponding to the LTS (n = 27 GCs). Surprisingly, the putative LTS does not follow an all-or-none rule but decreases with distance (n = 8 GCs). In the subthreshold regime, EPSPs summate on average linearly at the soma, with occasional supra- and sublinear summation, and Ca^{2+} signals in individual spines (n = 20 in 10 GCs) and in the dendrite (n = 14) increase with the total number of activated spines. In conclusion, GCs turn out to be yet more excitable than previously thought, and postsynaptic spine Ca^{2+} entry is tuned to the overall level of excitation. Thus the GC spines - who according to our previous findings can operate as independent mini-neurons – do sense the general activation state of their 'mother GC' already at subthreshold levels. Funded by the BMBF (01GQ1502).

SYMPOSIUM 6: INTEGRATIVE NEUROBIOLOGY IN METABOLISM

S25

The neurobiology of homeostasis

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The brain contains dedicated neural circuits that sense the physical needs of the body and then transform those needs into motivated behaviors such as eating and drinking. How this transformation is performed remains poorly understood. I will describe recent work from my lab investigating the cells, circuits, and dynamics that give rise to ingestive behavior.

S26

Vagus nerve and reward

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The gut is now recognized as a major regulator of motivational and emotional states. However, the relevant gut-brain neuronal circuitry remains unknown. It will be shown how the sensory branches of the vagus nerve, specifically, those innervating the upper gut, control food reward by modulating midbrain dopamine systems. Asymmetric central pathways of vagal origin will be outlined, including a brainstem relay linking the right vagal sensory ganglion to the dopamine cells of Substantia nigra. The implications of these findings for our current understading of brain reward pathways, including for the treatment of affective and eating disorders, will be discussed.

S27

Deciphering the neuronal control of systemic insulin sensitivity

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Over the last decades, our understanding of the fundamental homeostatic processes governing energy balance and glucose homeostasis has largely evolved and pinpointed a pivotal role of the central nervous system and more particularly of the arcuate nucleus of the hypothalamus (ARH). Within the ARH, activation of orexigenic AgRP-expressing neurons potently promotes feeding. We demonstrate that in addition to modulate feeding, chronically altering AgRP-neurons activity also affects peripheral glucose homeostasis. Further, acute activation of AgRP-neurons causes insulin resistance through impairment of insulin-stimulated glucose uptake into brown adipose tissue (BAT) and decreases sympathetic nerve activity. Optogenetic circuitry mapping reveals that feeding and insulin sensitivity are controlled by both distinct and overlapping AgRP-projections. Notably, we found that activation of AgRP → ventro lateral part of the anterior bed nucleus of the stria terminalis (aBNST)vl projections mediate the effect of AgRP neuron activation on insulin sensitivity. Collectively, our results suggest that AgRP neurons in mice induce not only eating, but also insulin resistance, revealing a mechanism by which these neurons rapidly coordinate hunger states with glucose homeostasis. Aiming to uncover new regulator of AgRP-neurons, we discover a novel AgRP-neurons' stimulatory pathway by demonstrating that they express the purinergic receptor 6 (P2Y6) and that activation of P2Y6 by its endogenous ligand uridine-diphosphate (UDP) increases AgRP-neuron's action potential firing and promotes feeding. We further show that selectively abrogating P2Y6-signaling in AgRP-neurons alleviates obesity-associated adiposity, hyperphagia and insulin resistance. Taken together, our work reveals that modulating AgRP-neurons by targeting P2Y6-signaling improves obesity and obesity-associated metabolic outcomes.

S28

Sympathetic neuroimmunity for obesity

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The brain controls adiposity via central and peripheral neural circuits. We used molecular genetic tools such as optogenetics to probe the connection between peripheral sympathetic neurons and adipocytes. Further, we found this neuro-adipose junction to drive lipolysis fat mass reduction via norepinephrine (NE) signaling (1). As obesity is a chronic inflammatory state, we set to define neuroimmune mechanisms that link inflammation to SNS neurons (2). We report the discovery of Sympathetic neuron-Associated Macrophages (SAMs) that directly regulate the extracellular availability of norepinephrine (NE). We identified the molecular mechanism by which SAMs import and metabolize norepinephrine (NE). Abrogation of the mechanism for the uptake of NE by SAMs increases NE availability, which in turn promotes thermogenesis and browning, and long-term amelioration of obesity independently of food intake (3). The role of SAMs at steady state and obesity will be discussed.

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SYMPOSIUM 7: CHEMORECEPTORS THAT MAINTAIN THE INTERNAL MILIEU

S29

Gingival solitary chemosensory cells serve as immune sentinels to protect against periodontitis

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Extra-oral taste-like solitary chemosensory cells (SCCs) mediate innate immune responses. SCCs utilize Tas2r "bitter" receptors and downstream "taste" signaling pathways to detect pathogens and their toxins, triggering host defenses. We have found SCCs in gingival tissue where they regulate the oral microbiome and protect against periodontitis. By RT-PCR we found expression in mouse gingival tissue of 10 Tas2rs along with Gnat3 (α-gustducin), Plcβ2 and TrpM5. By immunohistochemistry we showed that Gnat3, Plcβ2 and TrpM5 are co-expressed in gingival SCCs. Skn1a-/- mice lacking SCCs in other tissues were also devoid of gingival SCCs. Compared to wildtype (WT), Gnat3-/- mice had accelerated naturally occurring alveolar bone loss. Knockout mice lacking Gnat3, TrpM5 or Skn1a displayed enhanced ligature-induced periodontitis compared to WT controls. Compared to WT, Gnat3-/- mice had higher bacterial loads on their periodontal ligatures, up-regulated levels of pro-inflammatory cytokines and lower levels of antimicrobial peptides in their gingival tissues. 16S rRNA sequencing showed that the absence of Gnat3 alters the oral

microbiome, e.g. WT mice had higher levels of muribacter, while Gnat3-/- mice displayed higher levels of corynebacteria. Twice daily topical application to the gingiva of bitter denatonium benzoate for one week stimulated gingival production of defensins and reduced alveolar bone loss in WT but not in Gnat3-/- mice. In sum, mouse gingival SCCs likely respond to bacterial signals via Tas2rs and downstream taste signaling components to trigger host secretion of antimicrobial peptides and other innate immune responses to prevent overgrowth of pathogenic oral bacteria and regulate gingival microbial composition. Gingival SCCs may provide a useful target for treating periodontitis. Furthermore, the Tas2r genotype may provide an effective basis for personalized dental treatments against periodontitis.

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S30

Roles of sugar transporters and calorie sensors in taste cells

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Previous studies demonstrated that T1R2+T1R3, the primary sweet sensor for sugars and noncaloric sweeteners, is expressed not only in taste cells but also gut enteroendcrine cells and pancreatic beta cells. Activation of the sweet receptor by sugars and sweeteners, leads to facilitation of glucose absorption in the gut and insulin secretion in the pancreas. We showed that sweet sensitivities of these T1R3-expressing cells in the oral cavity and gut are inhibited by leptin, a satiety hormone, leading to decreased appetite and increased energy expenditure via the hypothalamus. Thus, by acting not only via the central nervous system but also peripheral sweet-responsive organs, leptin may play important roles in regulating energy homeostasis. By contrast, recent studies demonstrated that metabolic sensors (K_{ATP} channels) and glucose transporters (SGLTs/GLUTs) originally found in the gut and pancreas are also expressed in T1R3-expressing taste cells. Our electrophysiological studies showed that there exist sweet-responsive fibers of the mouse chorda tympani nerve whose responses to sugars, but not to noncaloric sweeteners, are enhanced by addition of Na^+ and the mixture responses are inhibited by Phloridin, an SGLT inhibitor, and sweet-responsive cells whose responses to sweet compounds are inhibited by leptin via K_{ATP} channels. These results suggest that the glucose transporter may act as a glucose sensor and forms a T1Rs-independent sweet taste pathway together with K_{ATP} channels in taste cells. The results of the above experiments will be described and functional roles of sugar transporters and calorie sensors in sweet-responsive cells will be discussed

S31

Regulations of neural circuits underlying body fluid balance

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Body fluid homeostasis is a vital function that regulates the balance between water and sodium. If this balance shifts in one direction, the brain detects the changes and triggers appetite to drive water or sodium intake for compensation. Defining the neural logic of these processes is critical for understanding brain function underlying normal and abnormal appetite. Our goal is to understand how the brain processes internal state information and peripheral signals to regulate goal-oriented behaviors. In this presentation, we use mice as a model organism to identify peripheral and central mechanisms of body fluid regulation. We will describe how imbalance between water and salt affects peripheral sensory detection and central neural activities, and how the brain processes these signals to drive consumption. We also use in vivo calcium recording, circuit mapping, and molecular tools to show that specific neural populations in the thirst circuits integrate peripheral sensory and homeostatic information to optimize drinking behavior.

S32

Taste receptors in hypothalamic tanycytes: central nutrient sensors and potential contributors to energy homeostasis

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Hypothalamic tanycytes are specialized glial cells that contact the cerebrospinal fluid (CSF) of the 3rd ventricle and have a long process that enters the brain parenchyma. Their specialized anatomical features have long suggested that they might sense the composition of the CSF and relay this information to the hypothalamic neurons that control food intake, energy expenditure and hence body weight. We have tested the hypothesis that tanycytes sense nutrients in the CSF. Fascinatingly, tanycytes possess a range of taste receptors (sweet, umami and bitter) and use these receptors to detect glucose, amino acids (both essential and non-essential) and bitter tasting substances (which includes the essential amino acids tryptophan and phenylalanine). In all cases,

activation of the taste receptors evokes a Ca^{2+} signal, the release of ATP and a secondary wave of Ca^{2+} mobilization triggered via ATP receptors. For glucose, this secondary Ca^{2+} wave is triggered via the P2Y1 receptor. For the amino acids, activation of additional ATP receptors is also required most probably of the P2X subtype. In a further similarity with the signal transduction pathways of taste buds, the ATP release is channel mediated. For arginine (detected via Tas1r1/r3), ATP release occurs via the pannexin 1 channel, whereas for alanine (detected via mGluR4), ATP release occurs via CaLHM1. Amino acids are powerful mediators of satiety and direct injection of amino acids into the brain will reduce food intake. As brain levels of amino acids vary with nutritive state, central amino acid detection is likely to be an important regulator of appetite. Our data strongly suggests, but does not yet show, that tanycytes could be key central mediators of satiety and have an anorexigenic action. The current emphasis of our research is to understand the behavioural and physiological significance of taste receptor-mediated nutrient sensing by tanycytes.

SYMPOSIUM 8: PLASTICITY IN CHEMOSENSORY SYSTEMS

S33

Plasticity of olfactory circuits in Drosophila melanogaster

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Animals use sensory systems to navigate the environment in a way that optimizes their survival and reproduction. The olfactory system plays here a key role in encoding chemical information and translating the outside world into a neuronal representation that enables an animal to take odor-guided decisions. The structure and function of the olfactory system of Drosophila is well characterized. However, little is known about whether and to which extent individual experience is modulating the olfactory circuitry. We therefore aim at elucidating plastic changes at the morphological, functional and behavioral level. Our recent studies have shown that the first olfactory neuropil, the antennal lobe, reveals a unique connectivity of input and output neurons for each individual glomerulus, which is correlated to behavioral relevance. Using tracing methods, neuronal reconstructions, functional two-photon calcium imaging and behavioral assays to monitor odor-guided behavior, we investigate the influence of experience and learning to specific odors after long-term exposure. The talk will summarize our recent insight into the plastic changes of the olfactory circuitry.

S34

Evolution of the male olfactory system in honey bees (genus Apis)

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All honey bee species (genus Apis) display a striking mating behavior with the formation of male (drone) congregations, in which virgin queens mate with many drones. Bees' mating behavior relies on olfactory communication involving queen- but also drone pheromones. To explore the evolution of the neural system involved in sex communication in honey bees, we analyzed the neuroanatomical organization of the antennal lobe (primary olfactory center) in the drones of five species from the three main lineages (dwarf honey bees: A. florea, giant honey bees: A. dorsata, cavity-nesting honey bees: A. mellifera, A. kochevnikovi and A. cerana) and from three populations of A. cerana (Borneo, Thailand and Japan). In addition to differences in the overall number of functional units, the glomeruli, our data reveal marked differences in the number and position of macroglomeruli, enlarged functional units putatively dedicated to sex pheromone processing. Dwarf and giant honey bee species possess 2 macroglomeruli while cavity-nesting bees present 3 or 4 macroglomeruli, suggesting an increase in the complexity of sex communication during evolution in the genus Apis. The three A. cerana populations showed differing absolute numbers of glomeruli but the same three macroglomeruli. Overall, we identified 6 putatively homologous macroglomeruli in the genus Apis. One of these, which is dedicated to the detection of the major queen compound 9-ODA in A. mellifera, was conserved in all species. We discuss the implications of these results for our understanding of sex communication in honey bees and propose a putative scenario of antennal lobe evolution in the Apis genus.

S35

The synaptic logic of associative olfactory learning in Drosophila

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Plastic changes in synaptic transmission are a neuronal substrate underlying learning and memory formation. However, it is challenging to determine which and how individual synaptic connections change to acquire a stimulus-specific memory. We used in vivo calcium imaging in Drosophila to

monitor learning-induced synaptic plasticity. Fruit flies can learn to avoid an odor stimulus that is temporally paired with a punitive electric shock. We trained fruit flies positioned under a two-photon microscope using this classical aversive olfactory conditioning regime, and monitored odor-evoked calcium activity through a window cut in the head capsule. One odor (CS+) was presented in coincidence with a punitive electric shock. A second odor (CS-) was subsequently presented without punishment. Control animals received the same odorant stimulation, but without the electric shock. The MARCM technique was used to express the calcium sensor GCaMP in single γ-lobe Kenyon cells of the mushroom body, a brain region to which the acquisition of associative olfactory short-term memory could be localized. We measured odor-evoked activity in synaptic boutons along individual axons and across many neurons. Albeit associative learning induced bi-directional changes in synaptic bouton activity, their overall calcium activity across all boutons remained constant. However, odor-evoked synaptic bouton activity within and across single Kenyon cells de-correlated as a result of associative learning, and specifically for the CS+. No de-correlation between boutons was observed for the calcium activity evoked by the CS- or the control odor. This reveals a novel principle of how associative memories can be differentially encoded across assemblies of neurons and axonal compartments. The finding and its implications will be discussed in the context of the neuronal circuitry underlying olfactory learning in Drosophila.

S36

The sugar taste of honeybees

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Honeybees (Apis mellifera) have a diet limited to floral nectar and pollen. Floral nectar is mainly composed of carbohydrates, whereas pollen is composed of amino acids and fats. Compared to other insects, the honeybee possesses fewer genes for gustatory receptors (Grs), implying bees have a limited ability to taste chemical compounds. The few studies of honeybee Gr genes are restricted to three: AmGr1, 2 & 3 and their expression in Xenopus oocytes suggests they encode sugar receptors (Jung et al., 2015; Takada et al., 2018). However, no study has assessed Gr function directly. Here we use an 'empty sugar neuron system' in Drosophila as a novel heterologous expression system to assess honeybee Gr function.

Gr genes were expressed in a Drosophila octuple mutant backgournd - lacking all 8 Drosophila sugar Gr genes. Within the empty sugar neuron, we expressed the predicted honeybee sugar Grs alone and in pair-wise combination, and measured neuronal responses to sugars using Ca+ imaging. Additionally, in vivo responses to sugars were assessed using tip recording electrophysiology from the galeal and labial palp sensilla of honeybees.

We find that multiple honeybee Grs play a role in sugar detection and interestingly, can potentially function as single proteins [homomultimers are possible]. Notably, AmGr3 − the ortholog to the Drosophila fructose receptor (DmGr43a) − appears to function in a more generalist manner, detecting glucose, maltose and melezitose in addition to fructose and sucrose. Generally, the sugars that evoked responses from multiple Grs also demonstrated the highest spiking rate from the honeybee mouthpart sensilla.

While highly tuned to sugar detection, some honeybee Grs appear to function as monomers or homomultimers, in contrast to the Grs of other insect species. Greater receptor generalisation may compensate for low receptor number, allowing forager bees to rapidly respond to their carbohydrate-laden landscape. Taste grant funded through the BBSRC.

LATE-BREAKING LECTURE

S37

The neuronal encoding of oral fat by the coefficient of sliding friction in the cerebral cortex and amygdala

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Fat in the diet contributes to the pleasant mouth feel of many foods, but overconsumption may contribute to obesity. The properties of fat in the mouth that are sensed was analysed using the responses of neurons in the macaque insular taste cortex, and two areas to which it projects the orbitofrontal cortex where the pleasantness of fat is represented, and the amygdala (Rolls, 2015). We discovered that the firing rate responses of these fat-responsive neurons are correlated with the coefficient of sliding friction, and not with viscosity which reflects food thickness. Other, not fat-sensitive, neurons encoded viscosity and not the coefficient of sliding friction. Neuronal population analyses confirmed that fat-responsive neurons conveyed information about the coefficient of sliding friction but not about viscosity. Conversely the viscosity-sensitive neuronal population conveyed information about viscosity, but not about the coefficient of sliding friction. Some of these neurons combined this oral texture information with information about taste (Rolls, 2015). This new understanding

of the representation of oral fat in the cerebral cortex and amygdala opens the way for the systematic development of foods with the pleasant mouth-feel of fat, together with ideal nutritional content, and has great potential to contribute to healthy eating and a healthy body weight (Rolls et al, 2018).

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SYMPOSIUM 9: ECRO YOUNG INVESTIGATORS

S38

Location of odorant-receptor interaction within OR1A1 binding cavity is crucial for their activation potential

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Human olfactory detection relies on odorant receptors (OR), which belong to the G protein coupled receptor (GPCR) family. The current hypothesis is that an odorant's percept is caused by a specific activation pattern of ORs. However, the structure-activity relationship between the odorant, the OR space and the rules behind the amazing smelling potential of some molecules remain partially unknown. Most odor molecules trigger OR activation with EC50 values around hundreds of µM in vitro, but some odorants possess the capability of triggering OR activation at much lower concentrations with EC50 around 1µM to 100nM.[1] Here, we use a transdisciplinary method combining functional assays, site directed mutagenesis and molecular modeling that previously shown to be efficient in resolving OR/odorants interactions[2] We aim to decipher the differences of binding in six agonists to the well-studied human OR, OR1A1, going from very strong (EC50=1µM) to weak agonists(EC50>316µM). We found that some residues within the binding cavity are crucial for the binding of strong agonists. This suggests that, in the canonical binding cavity of OR1A1, specific binding location may have a control on the ability of an Department of Evolutionary Neuroethology, Max Planck Institute for Chemical Ecology, Hans-Knoell-Str. 8, 07745, Jena, Germany

Evaluating odor blends in sensory processing is a crucial step for signal recognition and execution of behavioral decisions. Using behavioral assays and 2-photon imaging, we have characterized the neural and behavioral correlates of mixture perception in the olfactory system of Drosophila. Mixtures of odors with opposing valences elicit strong inhibition in certain attractant-responsive input channels. This inhibition correlates with reduced behavioral attraction. We demonstrate that defined subsets of GABAergic interneurons provide the neuronal substrate of this computation at pre- and postsynaptic loci via GABAB- and GABAA receptors, respectively. Intriguingly, manipulation of single input channels by silencing and optogenetic activation unveils a glomerulus-specific crosstalk between the attractant- and repellent-specific circuits. This inhibitory interaction biases the behavioral output. Such a form of selective lateral inhibition represents a crucial neuronal mechanism in the processing of conflicting sensory information.odorant to trigger its activation. Such strong agonists of OR have been occasionally linked to low detection thresholds[3], suggesting that understanding the ability of an odorant molecule to strongly activate ORs could help rationalize their smelling potential.

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S39

Three-dimensional reconstructions reveal a ventral glomerular deficit in parkinson's olfactory bulb

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Olfactory dysfunction is common in Parkinson's disease (PD) and is an early symptom, but its pathogenesis remains poorly understood. We report a quantitative approach to describe the human olfactory bulb, and statistical comparisons between olfactory bulbs from normal and PD cases. We subjected horizontal 10 µm sections of olfactory bulbs from six normal and five PD cases to fluorescence immunohistochemistry. We scanned the stained sections with a fluorescence slide scanner, segmented the glomeruli, and generated three-dimensional reconstructions of whole olfactory bulbs. We document the occurrence of atypical glomerular morphologies and glomerular-like structures deep in the olfactory bulb, both in normal and PD cases. We define a novel parameter: the global glomerular voxel volume (GGVV), which is the total volume of all voxels that are classified immunohistochemically as glomerular. We find that the GGVV of olfactory bulbs from PD cases is half of that from normal

cases. Moreover, the distribution of glomerular voxels along the dorsal-ventral dimension of the olfactory bulb is significantly altered in PD cases: whereas most glomerular voxels reside within the ventral half of the olfactory bulb from normal cases, glomerular voxels are more evenly spread among the ventral and dorsal half-bulbs from PD cases. These observations indicate a preferentially ventral glomerular deficit in PD, which is consistent with the olfactory vector hypothesis for its pathogenesis. The distribution of α -synuclein also correlates with that of glomerular voxels. Our quantitative approach will help our understanding of the human olfactory bulb and its alterations in PD.

S40

Odor mixtures of opposing valence unveil inter-glomerular crosstalk in the Drosophila antennal lobe

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Evaluating odor blends in sensory processing is a crucial step for signal recognition and execution of behavioral decisions. Using behavioral assays and 2-photon imaging, we have characterized the neural and behavioral correlates of mixture perception in the olfactory system of Drosophila. Mixtures of odors with opposing valences elicit strong inhibition in certain attractant-responsive input channels. This inhibition correlates with reduced behavioral attraction. We demonstrate that defined subsets of GABAergic interneurons provide the neuronal substrate of this computation at pre- and postsynaptic loci via GABAB- and GABAA receptors, respectively. Intriguingly, manipulation of single input channels by silencing and optogenetic activation unveils a glomerulus-specific crosstalk between the attractant- and repellent-specific circuits. This inhibitory interaction biases the behavioral output. Such a form of selective lateral inhibition represents a crucial neuronal mechanism in the processing of conflicting sensory information.

S41

Virus-assisted single-cell transcriptomics for the genetic dissection of neural circuits

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Animals employ instinctive behavioral and physiological responses to a variety of stressors to overcome danger and restore homeostasis. Hypothalamic corticotropin-releasing hormone (CRH) neurons govern the physiological response to stress by regulating the hypothalamic-pituitary-adrenal axis, which controls circulating levels of stress hormones. At present, the neural circuits and molecular mechanisms that convey different stress signals to CRH neurons are poorly understood. Here, we developed a novel strategy, termed "Connect-Seq," which uses single-cell RNA sequencing of virus-infected cells upstream of specific neurons in neural circuits to define their molecular identities. As a proof of concept, using Connect-Seq, we profiled single-cell transcriptomes of ~124 brain neurons and identified subpopulations of neurons that are likely to communicate stress-related signals to CRH neurons. Analyses of single-cell transcriptomes for 'fast-acting' neurotransmitters revealed subpopulation of cells expressing markers of inhibitory GABAergic neurons and excitatory glutamatergic neurons. Further analyses showed a number of other neuromodulators/neurotransmitters in upstream neurons, including acetylcholine, dopamine, histamine, norepinephrine, and, altogether, ~43 different neuropeptides, each expressed in individual neurons or subsets of neurons. These findings reveal extreme molecular heterogeneity among the upstream neurons and suggest they use diverse neurochemical messengers to transmit signals to CRH neurons. Many neurons coexpressed different neurotransmitters/neuromodulators, suggesting co-release of neurochemical messengers. Dual labeling of brain sections verified expression of specific neuropeptides in virus-infected neurons upstream of CRH neurons in selected brain areas. Our results indicate that Connect-Seq can be applied to genetically dissect neural circuits and uncover molecular identities of neurons upstream of specific neuronal types of known function.

S42

A crucial role of Trpc5 in maintaining infraslow membrane potential oscillations in hypothalamic dopamine neurons

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Olfactory sensory input can modulate endocrine events, e.g. prolactin secretion, which can lead to pregnancy failure. Its secretion is under inhibitory control by dopamine released from neuroendocrine cells in the hypothalamic arcuate nucleus (ARC). During early pregnancy, a decline in dopamine release is required to elevate prolactin levels, thereby stimulating a hormonal pathway that prepares the uterine endometrium for implantation of a fertilized ovum. Thus, the firing properties of these neurons control the release of dopamine and should therefore be critical to determine reproductive success. Here, we identify the canonical transient receptor potential (TRPC) channel Trpc5 as an essential requirement for normal function of dopamine ARC neurons and prolactin homeostasis. By analyzing female mice carrying targeted mutations in the Trpc5 gene, we show that Trpc5 is required for maintaining highly stereotyped infraslow membrane potential oscillations of dopamine ARC neurons. Trpc5 is also required for eliciting prolactin-evoked tonic plateau potentials in these neurons that are part of a regulatory feedback circuit. Trpc5 mutant females show severe prolactin deficiency or hypoprolactinemia that is associated with irregular reproductive cyclicity, gonadotropin imbalance, and impaired reproductive capabilities. These results reveal a previously unknown role for the cation channel Trpc5 in prolactin homeostasis of female mice and provide new strategies to explore the genetic basis of reproductive disorders and other malfunctions associated with defective prolactin regulation in humans.

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S43

Coordinated activity in the olfactory bulb gates the oscillatory entrainment of entorhinal networks in neonatal mice

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While the developmental principles of sensory and cognitive processing have been extensively investigated, their synergy has been largely neglected. Early in life, most sensory systems are still immature. As a notable exception, the olfactory system reaches full maturity during intrauterine life, controlling mother-offspring interactions and neonatal survival. Here, we elucidate the structural and functional principles underlying the communication between olfactory bulb (OB) and lateral entorhinal cortex (LEC) – the gatekeeper of limbic circuitry – during neonatal mouse development. Using genetic tagging of mitral/tufted cell (MTC) projections and retrograde tracing, we show that bilateral connectivity between OB and LEC is established at the end of the first postnatal week. Extracellular recordings in vivo from postnatal day (P) 8–10 pups reveal tight coupling of theta bursts (4–12 Hz) and continuous respiration-related activity (RR, 2–4 Hz) between OB and LEC. Combining optogenetics, pharmacology, and electrophysiology in vivo, we show that MTC-dependent discontinuous theta bursts in OB drive network oscillations and time action potential firing in LEC. Pharmacological silencing OB activity diminishes entorhinal oscillations, whereas odor exposure boosts OB-entorhinal coupling. These results demonstrate that the oscillatory coupling between OB and LEC emerges during early development. Spontaneous, coordinated activity of MTCs controls the frequency-specific network entrainment of neonatal LEC, thereby facilitating the maturation of limbic circuitry. The project was funded by the German Research Foundation (Ha4466/10–1, SFB 936 (B5)).

S44

A mirror-symmetric excitatory link coordinates odor maps across olfactory bulbs and enables odor perceptual unity

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Sensory input reaching the brain from bilateral and offset channels is nonetheless perceived as unified. This unity could be explained by simultaneous projections to both hemispheres, or inter-hemispheric information transfer between sensory cortical maps. Odor input, however, is neither topographically organized, nor does it project bilaterally, making olfactory perceptual unity enigmatic. Here we report a novel circuit that interconnects mirror-symmetric isofunctional mitral/tufted cells between the two mouse olfactory bulbs. Connected neurons respond to similar odors from ipsi- and contra-nostrils whereas unconnected neurons do not respond to odors from the contralateral nostril. This connectivity is likely mediated through a one-to-one mapping from mitral/tufted neurons to the ipsilateral anterior olfactory nucleus pars-externa which activates the mirror-symmetric isofunctional mitral/tufted neurons glutamatergically. This circuit enables the sharing of odor information across hemispheres in the absence of a cortical topographical organization, suggesting

that olfactory glomerular maps are the equivalent of cortical sensory maps existing in other senses.

S45

Smell-S and Smell-R: Olfactory tests not influenced by odor-specific insensitivity or prior olfactory experience

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[Aims] Smell test scores are strongly influenced by cultural factors and genetic background that determines sensitivity to a specific odor. This introduces sources of bias into most available smell tests. To overcome this, we created a smell sensitivity test (SMELL-S) and a resolution test (SMELL-R) based on complex odor-mixtures that should minimize these issues.

[Methods] We enrolled 75 healthy subjects who underwent a reliability study. Then, we invited 23 healthy subjects with variable sensitivity to phenylethyl alcohol (PEA) and 10 participants complaining of smell loss. They performed an accuracy study. Finally, 36 healthy Americans in New York City and 36 Taiwanese in Taichung participated to an equivalence study.

[Results] The reliability of SMELL-S and SMELL-R was high. The area under the ROC curve was 0.98 (95% CI=0.92–1.02) for SMELL-S and 0.83 (95% CI=0.69–0.97) for SMELL-R. The specificity of Sniffin' Sticks PEA threshold test and SMELL-S was 61.5% (16/26) and 100% (26/26), respectively. The difference between the Taiwanese and the Americans was much smaller for SMELL-R (v2) than the UPSIT, as determined by calculating the difference in z scores. SMELL-R mean score was significantly higher in the Taiwanese population.

[Conclusions] The proof-of-principle results suggest that SMELL-S and SMELL-R, which quantify the resolution and general sensitivity of the olfactory system, are reliable and accurate. SMELL-S overcomes genetic factors potentially biasing olfactory results. SMELL-R avoids the cultural bias seen for the UPSIT, in which test performance is systematically higher in the population for which the test was developed.

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SYMPOSIUM 10: MULTISCALE CHARACTERIZATION OF CHEMOPERCEPTION: FROM CHEMICAL COMPOUNDS TO SIGNALING NETWORKS

S46

Natural taste and mouthfeel modulators: Discovery inspired by culinary evolution

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The development of healthier food products, for example, reduced in fat, sugar, or salt, respectively, are well-known to induce nonacceptable flavor defects in the products and has, thus, created unexpected flavor challenges for the food industry. In response to the consumers' demand for healthy but tasty foods, novel ingredient discovery is essential to overcome such flavor challenges associated with the production of, in particular, sugar, salt or fat-reduced products.

Varying widely across the world, reflecting unique environmental, economic, and cultural traditions, various drying, fermentation, cooking and roasting procedures have been empirically developed during the last millenniums and, since then, the alluring flavor of the dishes prepared do attract consumers on a global scale. In particular, the food manufacturing techniques leading to the most premium tastes promise to contain essential taste compounds and/or taste modulators generated from sensory inactive precursors upon processing of the raw materials. This evolutionary refinement of food manufacturing procedures is, therefore, expected to open an interesting avenue towards the discovery of natural orosensory modulators, which might be applied as natural solutions to enhance culinary authenticity of convenience products or to overcome flavor challenges associated with the production of, in particular, sugar, salt or fat-reduced products. The presentation will highlight analytical strategies to identify taste and mouthfeel modulators in processed food by means of a Sensomics approach.

S47

Polypharmacology of bitter molecules

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Bitter taste presents an intriguing case of molecular recognition and functionality, since numerous, chemically diverse bitter compounds [1] are recognized by a varying number of T2R receptors.

Using chemoinformatics and machine learning tools, we found that T2R-promiscous agonists are typically smaller, more hydrophobic, and more globular than T2R-selective compounds [2]. Interestingly, the aversiveness [3] and toxicity [4] of T2R-promiscous and T2R-selective compounds do not seem to differ. hERG potassium channel, cytochrome P450 enzymes and carbonic anhydrases are common non-T2R targets of bitterants [5].

To expand the known bitter chemical space and to unravel common off-targets of bitterants, we developed BitterPredict machine learning tool [6], and the intense bitterness prediction tool (Dagan-Wiener et al., in preparation).

Prediction of polypharmacology for the bitter and intensely bitter molecules will shed light on bitter tastants function in oral and extra-oral tissues, and help unravel the molecular basis for discrimination between different bitter-tasting compounds.

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S48

Taste and chirality

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The importance of chirality in taste dates back to more than a century ago: in 1886 Arnaldo Piutti prepares D-Asparagine and discovers that it is sweet. In more recent times Solms and others (Bassoli et al., 2014) noted that amino acid enantiomers have different taste, mainly sweet for D-aa and bitter for L-aa. The relevance of chirality in the interaction of sweet substances with the receptor is dramatically shown by the difference between monellins built with L- or D- amino acids.

These observations are in the wake of many other observations on pairs of (non-optical) isomers with strikingly different taste: the examples of pairs of sweet/bitter isomers are numerous. In the past it was common belief that there had to be pairs of related sweet/bitter receptors.

The discovery of taste receptors shuttered many old beliefs, including the close relationship between sweet and bitter. I tried to reconcile old and new views by showing that the umami receptor could recognize some enantiomers and, by cross talk, convey a bitter sensation (Temussi, 2009). This hypothesis has been rejected by Meyerhof et al. (2015), albeit on the basis of too literal an interpretation.

Even if not a unique partner of the sweet receptor, the umami receptor can play a pivotal role in aftertaste.

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S49

The complexity of oral and extraoral tastant reception

Maik Behrens[1], Juergen Behr[1], Antonella Di Pizio[2], Kristina Lossow[3], Wolfgang Meyerhof[4], Masha Y Niv[2], Stefanie Nowak[5], Simone Prandi[5]

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The human sense of taste relies on chemosensory cells located in the oral cavity expressing subsets of a large variety of receptor molecules devoted to detect nutritionally relevant food-borne chemicals.

Frequently, even the decoding of taste properties of single compounds can be challenging, because of multiple, partially redundant receptive elements, gene variants and genomic arrangements. In fact, humans typically consume complex food items adding multiple layers of complexity. Moreover, detection of taste-active chemicals does not end in the oral cavity it continues along the gastrointestinal tract and is facilitated by the same set of receptors, but in different physiological

environments. Hence, to understand how tastants influence human physiology and health, a careful characterization of food-borne chemicals and their interaction with each other and with receptors from the oral cavity to the intestinal tract and beyond is necessary.

Using a range of methods including functional heterologous expression assays, molecular modeling and gene expression profiling, we investigated how taste receptors recognize multiple agonists, how compound mixtures can influence taste and how well the gastrointestinal tract is equipped with taste receptors for nutrient recognition.

SYMPOSIUM 11: NEW DEVELOPMENTS IN OLFACTORY SIGNALING

S50

Olfactory bulb changes in Parkinson's disease

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Six to ten years before a person visits the doctor with the cardinal features of Parkinson's disease (PD; paucity of movement and rigidity) they will usually have lost their sense of smell. Pathologically, PD is characterised by the accumulation of α-syn in various brain regions and a loss of dopamine cells in the substantia nigra. But, the olfactory bulb is one of the first brain regions affected by the accumulation of α-syn. Apparently, the majority of α-syn accumulates in neurons of the anterior olfactory nucleus in the olfactory bulb. α-syn spreads from the anterior olfactory nucleus to to the olfactory tract, anterior olfactory cortex and then into the substantia nigra. Thus, understanding the early changes in the olfactory bulb could be key in identifying early targets for slowing or stopping the progression of PD-related brain changes before the cardinal features appear. To this end we have performed studies on ethically sourced normal and PD human olfactory bulbs with a focus on characterising glomerular changes, measuring metal accumulation and identifying which cell types accumulate α-syn. To study glomeruli we have performed 3D reconstructions of whole human olfactory bulbs and have identified a ventral glomerular deficit and aberrant placement of many glomeruli in PD. To study metal accumulation we used inductively coupled plasma mass spectrometry, which demonstrated the regional distribution of metals in the bulb and in particular showed increased iron and sodium in the olfactory bulb in PD. Finally we have examined the cell types in which α-syn accumulates in the bulb. Our studies reveal that in PD bulbs, neurons, glia, pericytes and microglia all express α-syn in all cases studied. The results of these studies and implications for PD will be discussed.

S51

TAAR5 drives inter-male aggression independent of glomerular mapping

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The main olfactory system is required for proper social interactions and is activated by volatile social cues. However, f ew main olfactory receptors have been identified that contribute to specific social behaviors. The Trace Amine-Associated Receptors (TAARs) have been associated with presumptively innate responses to specific amines, including aversion to the kairomone phenylethylamine and attraction to the male urinary odor trimethylamine. Trimethylamine is enriched in the urine of sexually mature male mice, but the ethological relevance of this odorant is unknown. We show that trimethylamine elicits state dependent attraction or aversion in male and female mice, depending on neuroendocrine or social status. Subordinate males are averse to trimethylamine, while dominant males are attracted. Diestrus females are averse to trimethylamine, while estrus females are indifferent. Genetic knockout of TAAR5 abolishes valence responses in both sexes, and significantly reduces intermale aggression and aggression-induced defensive behaviors. Interestingly, loss of aggression from TAAR5 deletion can be rescued by broad overexpression of TAAR5 to ectopic glomeruli scattered throughout the olfactory bulb. A similar rescue via remapping of TAAR4 glomeruli is seen for aversion to phenylethylamine. Overall, the data identify TAAR5 as the first-identified main olfactory GPCR that mediates a specific social behavior in mice, and indicate that TAAR-mediated behaviors are independent of specific glomerular organization.

S52

Olfr78 is not required for oxygen regulation of breathing in mice

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The carotid body is essential for the adaptation of mammals to environmental or pathological conditions that result in hypoxemia. The carotid body contains neuron-like O2-sensitive glomus cells. In response to hypoxia, these cells release neurotransmitters that rapidly activate afferent sensory fibers stimulating the respiratory center and inducing hyperventilation. The mechanisms that glomus

cells utilize to detect changes in blood O2 tension have remained unclear. Single dissociated glomus cells can respond robustly to hypoxia when superfused with standard, lactate-free hypoxic solutions. As such, it was surprising that Chang et al. Nature 2015 claimed that lactate activation of an odorant receptor (Olfr78), which is expressed in glomus cells, is required for oxygen regulation of breathing. We found that our Olfr78 knockout mice (Bozza et al. Neuron 2009), which were also used by Chang et al. Nature 2015, have a normal hypoxic ventilatory response. The physiological responses of single glomus cells to hypoxia and lactate are indistinguishable between wild-type and our Olfr78 knockout mice.

S53

The discovery of bacterial signal peptides: from ligands to functions

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This lecture will focus on our current knowledge about possible interactions between the bacterial microbiome and chemosensory detection mechanisms in the mouse nose. The ability to detect specific chemical signatures released by microorganisms is a key function of host immune defense against pathogens. Such chemosensory processes not only occur in immune cells but also at many other places inside the body. Neither the underlying cellular and molecular mechanisms nor the detected ligands have yet been elucidated. We recently proposed that detection of formylated bacterial signal peptides by vomeronasal sensory neurons expressing formyl peptide receptors (Fprs) contributes to this process. These peptides represent a novel and structurally versatile class of Fpr activators. Fprs have a well-documented capability to interact with a variable set of agonists in a combinatory manner. This makes it difficult to elucidate the biological function of specific bacterial signal peptides and individual Fprs in different tissues. Nonetheless, a combination of high-throughput receptor pharmacology, molecular modeling together with cellular physiology and cross-species comparisons enabled us to make significant progress in understanding the role of individual Fprs for the detection of different types of peptides. We will here discuss our current concepts on the contribution of specific types of bacterial signal peptides to pathogen detection in chemosensory cells.

S54

How sensitive can you get? – Tracing amines and odorants to their receptors.

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The assignment of cognate odorant/agonist pairs is a prerequisite to understand odorant coding at the receptor level. For decades, this has been a challenging task, due to a rather sub-optimal plasma membrane expression of odorant receptors (ORs), as compared with other G protein-coupled receptors. We introduced a bi-functional N-terminal tag, called 'IL-6-HaloTag®', which facilitates functional cell surface expression of recombinant OR, and enables the quantification of cell surface receptor expression by live-cell flow cytometry. Typically, and depending on the cell line used, we observe an about four-fold increased surface expression, four- to fourteen-fold higher signaling amplitudes in the GloSensor â cAMP assay, and a significant higher potency of odorant-induced cAMP signaling as compared to the commonly used Rho-tag-OR constructs. Moreover, using IL-6-HaloTag® receptor constructs in the GloSensor â cAMP assay, now enables us to measure an activation of recombinant trace amine-associated receptors (TAARs) by their ligands in the Pico to Nano molar range. In summary, the combination of IL-6–HaloTag®-receptor constructs with the GloSensor â cAMP assay represents a powerful tool for the ligand identification and pharmacological characterization of chemosensory receptors.

SYMPOSIUM 12: CHEMOSENSORY PROCESSING IN INSECT DISEASE VECTORS

S55

Evolution of signals and reception in blood feeding arthropods

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Signals and reception evolve in synchrony, and this reciprocal evolutionary relationship in chemical communication is broadly termed as 'co-evolution' to describe the evolution of secondary host metabolites and the accompanying step-wise adaptive responses by insects. Hematophagous arthropods successfully exploit vertebrates for blood feeding. This recent evolutionary shift demanded extensive neuroethological adaptations that are evident across the evolutionary range of vector species, from primitive ticks to advanced dipterans. Senses are biological features that are shaped by natural selection to promote adaptive behavior,

thus a variety of exciting patterns are apparent in what they sense and how. Hematophagous arthropods display robust olfactory driven behaviors. A distinct yet limited range of volatile organic compounds are parsimoniously used as cues for locating hosts and habitats, or, to avoid the unsuitable ones. These chemicals elicit behaviors such as attraction or repulsion/avoidance while vectors seek habitats, hosts, mates, or oviposition sites. I will present data from extensive analyses of the chemical landscape that define the odor space of these arthropods. Additionally, data from the structural and functional studies of their olfactory systems and insights from our recent genomic sequencing efforts will be presented.

S56

Developing push-pull strategies for surveillance and control of Malaria vectors

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[Introduction] Malaria transmission continues despite the widespread use of long-lasting insecticide-treated bed nets (LLINs) and case management. Early evening and outdoor mosquito biting and the development of insecticide resistance threaten long-term progress towards the goal of malaria eradication. Using non-pyrethroid spatial repellents in combination with odour-baited mosquito traps we developed a push-pull system to repel (push) malaria vectors away from the peridomestic environment and pull them in to traps, thus removing them from the environment and reducing biting risk.

[Objectives] To determine whether our novel push-pull technology leads to reduced mosquito house entry rates and lower human landing rates.

[Methods] Experiments were conducted under semi-field and field conditions in western Kenya. In semi-field systems, human landing rates of unfed Anopheles arabiensis were measured when push-only (para-menthane-3,8-diol (PMD)), pull-only (Suna traps) and combined push-pull systems were in place. In the field we monitored mosquito house entry rates when push-only (delta-undecalactone (dUDL)), pull-only (Suna traps) and combined push-pull systems were in place.

[Results] Push-pull using PMD was not associated with any reduction in human landing rate under semi-field conditions. In contrast, push-pull using dUDL in the field led to 50% lower house entry rates by An. gambiae s.l. and An. funestus compared to the control setting where only bed nets were used (N = 1248 trap nights, $P < 0.05$).

[Conclusions] Push-pull is a promising new tool for the control of malaria which should be effective in regions where vectors are insecticide resistant and bite outside the times and spaces where LLINs are used. Further studies will model the possible epidemiological impact of push-pull on malaria transmission.

Sources of funding: Bill and Melinda Gates Foundation, Innovative Vector Control Consortium

S57

Effects of temperature on olfactory behavior in mosquitoes

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In order to obtain a blood meal, disease vector insects need to accurately identify and locate mobile vertebrate hosts using a wide range of cues, in particular olfactory signals, which are key mediator of the vector-host interaction. While the physiological processes regulating food-seeking behavior have been well studied in these insects, comparatively less is understood about how environmental temperature might affect the performance of their sensory system. This project aims at contributing closing the knowledge gaps in our understanding of thermal sensitivity in the disease vector mosquito Aedes aegypti.

S58

Chemosensory circuits driving Aedes aegypti attraction to humans

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The mosquito Aedes aegypti is a prolific vector of many arboviral diseases of global health significance including dengue and Zika. To orient towards humans from a distance to blood feed, Ae. aegypti is thought largely to rely on its sensitivity tuned sense of smell to track plumes of odorants present in body odor and human breath. To gain insight into the molecular and cellular basis of this sensory process, we have recently constructed a revised anatomical map of the Ae. aegypti antennal lobes (ALs) significantly expanding upon the number of annotated glomeruli previously described in this species. Using this approach, we have determined that the ALs of Ae. aegypti are arranged

in a stereotypical pattern in both male and female mosquitoes, although sexual dimorphism in glomerular volume, shape and number are observed. Furthermore, the digital in vitro AL atlas we have generated appears conserved across three different geographic strains of this mosquito species. Progress towards using this high-resolution in vitro AL atlas coupled with activity-dependent labeling approaches to decode mosquito sensory biology will be discussed.

S59

Modulation of host-seeking behavior in Aedes aegypti mosquitoes

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Olfactory learning in blood-feeding insects, such as mosquitoes, could play an important role in host preference and disease transmission. However, standardized protocols allowing testing of their learning abilities are currently lacking, and the molecular basis of their ability to form aversive memories remains unknown. Using a Pavlovian conditioning paradigm, we trained individuals Aedes aegypti mosquitoes to associate an odorant conditioned stimulus (CS), with a mechanical shock mimicking host defensive behavior (unconditioned stimulus; US). Results showed that learned information could be used by mosquitoes in a variety of experimental contexts. Molecular techniques were used to assess the role of dopamine in the formation of these aversive memories. Together, these results show that learning is a critical component in odor responses in Ae. aegypti, and provide the evidence for the functional role of dopamine for aversive learning memory in mosquitoes.

POSTER PRESENTATIONS

THEME I – INSECT CHEMORECEPTION

P1

Hawkmoth pheromone transduction employs a metabotropic cascade activating TRP-like ion channels while Orco controls response thresholds

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Insect odor transduction is under debate. Since insect olfactory receptors (ORs) are inversely inserted into the membrane of the dendritic cilia, it was questioned whether they can couple to G proteins in a metabotropic transduction cascade. Instead, it was suggested that ORs heteromerize with Orco, a spontaneously opening ion channel in olfactory receptor neurons (ORNs). It was hypothesized that ORs and Orco form an odor-gated receptor-ion channel complex, constituting the primary transduction channel that underlies odor responses in insects. However, in the hawkmoth Manduca sexta Orco did not open during the first 1000 ms of pheromone responses. Instead, Orco opens voltage-dependently, only during the late, long-lasting pheromone response. Thus, Orco appeared to control the membrane potential and, thus, the threshold of pheromone responses. Here, we further examined the role of Orco. Furthermore, we searched for ion channels underlying the primary events of pheromone transduction in vivo. In in vivo tip-recordings of pheromone-sensitive trichoid sensilla of hawkmoth antennae we found that spontaneous activity of ORNs expressed circadian rhythms that depended on Orco. Next we examined the mechanisms of circadian control of Orco testing its dependence on cyclic nucleotides. In addition, we found that pheromone responses activated G-protein-dependently a phospholipase C, hydrolyzing phospholipids, and generating diacylglycerol (DAG) and inositol-trisphosphate. With DAG analogs we found evidence for at least two different transient receptor potential (TRP)-like ion channels that underlie primary events of hawkmoth pheromone transduction. We conclude that hawkmoth pheromone transduction uses a metabotropic cascade involving activation of phospholipase C, opening TRP-like ion channels as primary transduction channels. Thus, hawkmoth pheromone transduction appears to resemble pheromone transduction in the vertebrate vomeronasal organ. [DFG grants STE 531/20-1,2 to MS]

P2

To sense or not to sense? Response of ORs to subthreshold stimuli

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Olfaction plays a central role for insects, as it provides essential information about food sources, predators and conspecifics. Volatile chemical cues in the environment are detected even at low concentrations by a specific class of olfactory receptors, the odorant receptors (ORs). The functional unit of these receptors is constituted of a heteromer formed by a specific ligand-binding OrX and a co-receptor called Orco.

It has been shown that ORs are capable of sensitization, meaning that repetitive subthreshold odorant stimulation

can elicit a response in Olfactory Sensory Neurons (OSNs). This was seen for Orco homomers and one OR in ex-vivo experiments and for three different sensilla in SSR. However, whether sensitization is a general property of ORs and its mechanism remains elusive.

In the present investigation we aim to answer these questions by means of calcium imaging techniques and pharmacology in an antenna ex-vivo preparation of the fruit fly Drosophila melanogaster.

P3

Candidate pheromone receptors of codling moth Cydia pomonella respond to pheromones and kairomones

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Olfaction plays a dominant role in the mate-finding and host selection behaviors of the codling moth (Cydia pomonella), an important pest of apple, pear and walnut orchards worldwide. Antennal transcriptome analysis revealed a number of abundantly expressed genes related to the moth olfactory system, including those encoding the olfactory receptors (ORs) CpomOR1, CpomOR3 and CpomOR6a, which belong to the putative pheromone receptor (PR) lineage, and the co-receptor (CpomOrco). Using heterologous expression, in both Drosophila olfactory sensory neurons and in human embryonic kidney cells, coupled with electrophysiological recordings and calcium imaging, respectively, we characterize the basic physiological and pharmacological properties of these receptors and demonstrate that they form functional ionotropic receptor channels. Both the homomeric CpomOrco and heteromeric CpomOrco + OR complexes can be activated by the common Orco agonists VUAA1 and VUAA3, as well as inhibited by the common Orco antagonists amiloride derivatives. CpomOR3 responds to the plant volatile compound pear ester ethyl-(E,Z)-2,4-decadienoate and the analogous methyl-(E,Z)-2,4-decadienoate, while CpomOR6a responds to the strong pheromone antagonist codlemone acetate (E,E)-8,10-dodecadien-1-yl acetate. Our previous investigations suggested CpomOR1 as the most promising candidate receptor for codlemone (E,E)-8,10-dodecadien-1-ol, the main pheromone the of codling moth.

Our findings represent important breakthroughs in the deorphanization of codling moth pheromone receptors, as well as more broadly into insect ecology and evolution and, consequently, for the development of sustainable pest control strategies based on manipulating chemosensory communication.

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P4

Molecular elements of chemosensation in antennae and palps of the desert locust

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The desert locust Schistocerca gregaria is endemic in Africa, the Middle East and parts of Asia, where locusts are feared due to their potential to swarm and devastate agriculture. The hemimetabolous locusts are characterized by a population density dependent phase polyphenism, including a solitary and a gregarious phase. The phase change does involve the perception of behavioral relevant semiochemicals, which are detected by specialized sensory cells, mainly located on the two primary chemosensory organs the antennae and mouthpart palps (maxillary and labial). On both organs we have identified odorant receptors (OR) and odorant-binding proteins (OBP), essential elements of the insect chemosensory system. A relatively high number of 119 ORs were identified from an antennal transcriptome of S. gregaria. Analyzing the receptors in more detail we found a number of ORs to be co-expressed with the "sensory neuron membrane protein 1", a marker for pheromone-sensitive neurons in holometabolous insects. Interestingly, compared to the higher number of ORs, only a small number of 14 OBPs were identified in the locust antennal transcriptome. The OBP-subtypes were found to be expressed in distinct subsets of cells in the four antennal sensilla types. Only three OBPs were specifically expressed in the OR-expressing sensilla basiconica and sensilla

trichodea. Analyzing the expression of OBPs on the labial and maxillary palps revealed that a subset of the OBP repertoire was also expressed on both palps, including OBPs found in OR-expressing sensilla of the antennae. Exploring the topographic expression pattern of the OBPs revealed a specific expression of OBPs in sensilla basiconica as well as in sensilla chaetica.

P5

Structure and function of individual projection neurons and centrifugal neurons in the male moth brain

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In the insect brain, different types of neurons provide input and output to the primary olfactory center, the antennal lobe (AL). The principal output neurons, projection neurons (PNs), convey odor information from the AL to higher brain areas by passing in one of six parallel antennal-lobe tracts (ALT). In turn, centrifugal neurons (CNs) innervating higher brain areas provide input to, and modulate, the processing in the AL. However, the functional role of the parallel tracts and CNs in olfactory processing, is yet poorly understood. Here, we have investigated these two types of neurons in the noctuid moth H. armigera, by using in vivo intracellular recording and iontophoretic staining in combination with confocal microscopy. Most of the PNs were confined to the three classic ALTs, the medial (m), mediolateral (ml), and lateral (l) ALT, but we also found neurons passing along two additional ALTs. Comparison of output areas of pheromone-sensitive PNs versus plant-sensitive PNs showed no overlap in the lateral protocerebrum. This applied not only to the m-ALT, but also to the ml-ALT and l-ALT. In addition, the results demonstrated that morphologically diverse PNs show different physiological response patterns, which suggests that individual ALTs are associated with distinct functions. Medial-tract PNs were narrowly tuned, indicating a function related to encode odor identity, whereas ml-ALT and l-ALT PNs were more broadly tuned suggesting other roles. In addition to the different categories of PNs, we also classified two types of CNs. One novel type, called the bilateral paired centrifugal neuron, responded to olfactory stimuli, indicating a role as feedback neuron. Overall, these data, which were obtained during a master project, provide an overview of the parallel ALTs connecting the antennal lobe with higher protocerebral regions. The findings contribute to improved characterization of not only antennal-lobe output neurons but also centrifugal neurons.

P6

Morphological and physiological properties of pheromone-sensitive projection neurons confined to parallel tracts in the male moth brain

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Previous data from mass staining experiments have demonstrated that macroglomerular complex (MGC) output neurons project along all three classical antennal-lobe tracts (ALTs), the medial, mediolateral, and lateral ALT (Homberg et al. 1988). However, except for medial-tract projection neurons (PNs), little is known about the individual neurons in the other tracts. In this study, we identified single male-specific PNs confined to all three ALTs in the brain of the moth, Helicoverpa armigera, by performing intracellular recording/staining combined with confocal microscopy. Several new types of MGC PNs were obtained. We also performed double anterograde labeling of the PNs from MGC and ordinary glomeruli (OG), respectively. The results demonstrated that there was a clear spatial segregation between MGC PNs and OG PNs in all tracts. Furthermore, all the lateral-tract MGC PNs were found to terminate in the column. In addition to the electrophysiology, we performed calcium imaging experiments for measuring odor-evoked responses from populations of MGC output neurons confined to different ALTs. Two types of staining procedures were carried out. One implied application of the calcium-sensitive dye to the calyces, leading to staining of the medial-tract PNs exclusively, whereas the other included dye application to the column region, resulting in labeling of PNs in lALT and mlALT. The data from the two categories of stained preparations confirmed the electrophysiological data. Thus, it appeared that the PNs confined to mALT are more sensitive and more narrowly turned than the PNs confined to lALT and mlALT. However, medial-tract PNs displayed a longer response latency (about 1 00 ms) than the group of PNs confined to non-mALTs. In summary, the morphological and physiological data obtained suggested district functions of the parallel pathways in pheromone signal processing.

P7

From the Labial Pit Organ to central nervous system: Complete mapping of CO_2 sensory neurons in the noctuid moth, Helicoverpa armigera

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As in other noctuid moths, specialized sensilla housed inside the labial pit organ (LPO) detect atmospheric carbon dioxide (CO_2) in Helicoverpa armigera. Previous studies have reported that the sensory neurons in these sensilla project to three main parts of the central nervous system: 1) the labial pit organ glomerulus (LPOG) in both antennal lobes, 2) the gnathal ganglion (GNG), and 3) the ventral nerve cord (Kent et al. 1986; Zhao et al. 2013). Whereas the main target area of the sensory neurons, i.e. the LPOG in the antennal lobe, has been thoroughly described previously, less is known about the two remaining target regions. In this study, we performed mass staining from the LPO combined with confocal scanning for analyzing more thoroughly the CO_2 projections in the brain and the ventral nerve cord. The detailed projection patterns in the GNG, described for the first time, show a dense network of thin terminals targeting the ipsilateral region, close to the midline of the ganglion, plus a few processes in the antennal mechanosensory and motor center (AMMC). The projection pattern in the ventral nerve cord, described for the first time as well, demonstrates relatively extensive processes in the ipsilateral part of the first thoracic ganglion. In addition, few axons innervate the ipsilateral part of the second thoracic ganglion.

To investigate putative overlap of sensory axon terminals originating from the LPO and distinct appendages, respectively, we performed additional double-labeling experiments by applying different dyes to 1) the flagellum of the antenna and the LPO and to 2) the tarsi and the LPO. Besides, we utilized an AMIRA reference brain for comparing target regions of sensory neurons originating from the antennal flagellum with those of LPO sensory neurons. None of these experiments revealed any overlap in the central nervous system.

P8

Application of supervised machine learning for understanding how input from odor mixtures is processed in the primary olfactory center

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Neural mechanisms underlying information processing of complex odor input is still poorly understood. By utilizing a super-smeller, the noctuid moth Helicoverpa armigera, we intend to explore computational principles typifying odor mixture processing in the insect's primary olfactory center, the antennal lobe. Our recent morphological studies of central olfactory pathways in the moth, demonstrated that the connection between the antennal lobe and the mushroom body calyx consists almost exclusively of uniglomerular projection neurons (PNs) confined to the medial antennal-lobe tract. This fact enables investigation of one output neuron category specifically. Thus, by applying a calcium indicator into the calyx region, the antennal-lobe dendritic parts of uniglomerular PNs will be labeled via retrograde transport. This, in turn, allows for measuring odor-evoked responses in this prominent population of antennal-lobe PNs.

The previous calcium imaging studies on retrogradely stained uniglomerular PNs demonstrated that this method is highly suitable for collection of big biological data reflecting odor coding within the antennal lobe neural network. Ongoing calcium imaging measurements will provide a data material enabling machine-learning analyses. Based on this data material, algorithms predicting which set of glomeruli will respond to a given odor mixture, and vice versa - which odor stimuli will elicit activity in distinct sets of glomeruli, will be established. An additional intermediate task is to generate a tool for identification of distinct glomeruli across different individuals. By characterizing the glomerular units physiologically and spatially across individuals, a machine-learning approach will be used to create a universal catalog of glomeruli.

P9

Spike characterization of local interneurons and projection neurons confined to parallel antennal-lobe tracts in the male moth brain

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The antennal lobe (AL) consists of numerous glomeruli formed by synapsis between sensory neurons and two types of central neurons: local interneurons (LNs), connecting the antennal-lobe glomeruli, and projection neurons (PNs), carrying odor information to higher centers. The PNs include several subtypes projecting via parallel antennal-lobe tracts, including the medial, mediolateral, lateral, and transverse tract. The purpose of the study presented here, is to investigate whether the morphologically distinct types of AL neurons, including LNs and all PN subtypes, are different and identifiable based on their spontaneous spiking patterns. By using the intracellular recording and staining technique in the AL of Helicoverpa armigera, we gathered physiological and neuroanatomical data on 41 LNs and 88 PNs. Non-parametric test statistics demonstrated several significant differences between the neuron categories, including distinctions between PNs from different tracts, and between LNs

and PN subtypes. The lateral-tract PNs differed significantly from one or more of the other neuron categories in respect to several interspike-interval based parameters. For instance, the rank of individual lateral-tract neuron`s average Poisson surprise was significantly higher than that of LNs (15.43 ± 5.41 vs. 11.69 ± 2.12, p = .03), all test statistics accompanied by median ± QIR. Moreover, the duration of the minimum burst fired by the neurons confined to the lateral tract was shorter than that fired by the LNs (25 ± 14 vs. 40 ± 7.25 ms, p < .01). The medial-tract PNs had a higher percentage of bursts and coefficient of variation than LNs (51.38 ± 21.32 vs. 22.29 ± 12.83 ms, p < .001; 1.24 ± 0.23 vs. 0.82 ± 0.43 ms, p = .03, respectively), which coincides with previous reports by Lei et al. (2011). The transverse- and mediolateral-tract PNs did not differ significantly from any other neuron category; these PNs rather appeared to resemble the LNs on several of the tested parameters.

P10

Neuronal response latencies provide a first general odor code in honeybees

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Odor stimuli are coded in the primary olfactory processing centers by spatiotemporal patterns of glomerular activity. The spatial distribution of odorant-induced responses has been extensively studied and was found to form a general odor code, conserved across individuals. However, the importance of temporal features for odor coding is still debated, and the universality of a temporal code remains unclear. Here, we studied the early dynamics of the odor code in the honeybee antennal lobes via fast two-photon calcium imaging. We find that the first information on the odorant quality is encoded in the relative firing onset latencies of antennal lobe output neurons. We show that these latency ranks form a universal code, conserved across subjects. Latencies allow to blindly predict an odorant stimulus with the same accuracy as the static response amplitude code. Indeed, glomerular latencies and amplitudes appear to encode complementary stimulus information. Finally, the latency code is in very good agreement with behavioral data obtained from an odor generalization assay. Tests on the discrimination of different odor concentrations instead show that the latency rank code is concentration-independent. These results show that neuronal response latencies provide the first information for odor identification, anticipating the slower amplitude code.

P11

Molecular basis of sugar perception in honeybees

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The perception of sugars such as sucrose or fructose is of highest importance for the evaluation of food sources by honeybee foragers. The sweetness of a nectar source is of paramount importance for the nectar collectors, but the contributions of the different sugars to the nectar is also important. Sugar evaluation is not restricted to foragers. Nurse bees utilize the stored honey for producing brood food and therefore should also be able to evaluate sugar sources. In fact, the individual evaluation of sugar stimuli is assumed to play a major role in division of labor within a honeybee colony. Our data show that foragers and nurse bees differ in their sensory responsiveness for two important sugars, sucrose and fructose, with foragers being more responsive. Independent of the social role, bees were less responsive to fructose than to sucrose. As responsiveness to sucrose correlates with responsiveness to fructose, we suggest a common regulation for the evaluation of sugar stimuli in the brain or the periphery. Intriguingly, the lower responsiveness to fructose of nurse bees compared to foragers correlates with a lower expression of the putative fructose receptor AmGr3 gene. These findings indicate a close connection between the expression of sugar receptor genes, responsiveness to sugar and social organization in the honeybee colony.

P12

Modulation of neuronal processing and behavior due to uni- and multi- modal learning in the honeybee Apis mellifera

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During their daily foraging flights, honeybees have to process environmental stimuli of different modalities, like vision, e.g. colorful petals, and olfaction, e.g. flower bouquets. From a morphological perspective both, the visual and the olfactory neuronal pathway converge at the calyx, the input region of the mushroom body (MB), a higher order brain center also involved in memory formation. Each MB is formed by about 170,000 Kenyon cells (KC). The Calyx is organized in different layers, each mainly innervated by one sensory modality. The KCs converge in approximately 400 MB-output neurons (MBON), which form several clusters near the vertical lobe. When we exposed the bee to olfactory, visual and

olfactory-visual (OV) compound stimuli during recording MBON activity we found four types of response behaviors in MBONs. MBONs sensitive to light only (i), sensitive to odors only (ii), sensitive to light and odors (iii), and MBONs not responding to any of the presented stimuli (iv). This suggests, that the layered input of the MB is conserved in a population of MBONs (i, ii), whereas a substantial proportion of MBONs integrate olfactory and visual information across MB input layers (iii). The not-responding population of MBONs (iv) may become recruited after a classical conditioning experiment as it was shown by Strube-Bloss et al. (2011). We therefore propose that reward association to an OV compound stimulus may recruit initially non sensitive MBONs which will encode the OV-reward association during memory retention.

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P13

Physiological, behavioural and developmental responses to host metabolites in a specialised herbivorous beetle

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Plant-feeding insects rely mainly on their gustation to decode the chemical composition of potential hosts. In close range, beetles for example scan leaves with their tarsi, mouthparts and antennal tips that are covered with e.g. gustatory sensillae. Gustatory receptor neurons that reside in such uniporous sensillae mediate the identification of feeding stimulants and deterrents from plants. Whereas generalist feeders are often deterred by plant secondary metabolites, the same nonvolatile ligands stimulate feeding of specialists. However, little is known about the perception of plant-derived tastants in specialised herbivorous beetles, despite their ecological and agricultural importance. One such intriguing example is the poplar leaf beetle Chrysomela populi that even sequesters the secondary metabolite salicin from its host (poplar leaves) into its body for use in defence. Here, we identified gustatory sensillae at the antennal tip of C. populi via scanning electron and confocal laser microscopy. The gustatory function of these sensilla chaetica was confirmed by single sensillum recordings using salt, sucrose and salicin. These primary and secondary metabolites were found at higher concentrations in methanolic leaf extracts of poplar compared to willow as control (which is only consumed when there are no alternatives). Feeding choice assays using these single metabolites or leaf discs showed preference for poplar over willow. Finally, we showed that these gustatory cues benefit the beetle's performance since weight gain was higher when C. populi was reared on leaves of poplar compared to willow. Overall, our study indicates that C. populi prefers feeding on the host that provides optimal development by tasting some of the host's abundant primary and secondary metabolites.

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P14

The expression patterns of SNMP1 and SNMP2 underlines distinct functions of two CD36-related proteins in the olfactory system of moths

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In moths, reproductive behavior largely depends on female- or male-released pheromones and their sensitive and accurate detection by conspecifics. Previous studies have shown that pheromone-responsive olfactory sensory neurons (OSNs) are characterized by expression of the "sensory neuron membrane protein 1" (SNMP1) that is supposed to function as co-receptor involved in transferring pheromones to adjacent pheromone receptors. In accordance with this notion, our studies revealed co-expression of SNMP1 and receptors for female sex pheromone components in OSNs of the moth Heliothis virescens. In addition, we found that support cells (SCs) of pheromone-responsive sensilla express the related protein, SNMP2. Like SNMP1, SNMP2 belongs to the CD36-family of two-transmembrane domain receptors and transporters for lipophilic compounds. Towards a better understanding of the role of the two SNMPs, we generated specific antibodies and conducted an in-depth analysis of their localisation in the antenna of male and female H. virescens. In line with a function in pheromone detection, SNMP1 was immunolocalized in the somata and the dendrites of OSNs in subsets of trichoid sensilla. The latter ones generally contained one SNMP1-positive OSN in males and clusters of 2–3 labelled cells in females. Immunhistochemical experiments with SNMP2-antibodies revealed a broad expression of this

protein in SCs of likely all trichoid and basiconic sensilla in adults. More detailed confocal and electron microscopic examination of olfactory sensilla located SNMP2-like immunoreactivity close to the apical membrane of SCs and interestingly inside the lymph space of trichoid and basiconic sensilla. This expression pattern suggests a more general function of SNMP2, possibly in processes related to clearance of olfactory sensilla. Together, our data emphasize distinct functions of the two SNMPs in the olfactory system of H. virescens.

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P15

Synaptic plasticity of olfactory microglomeruli in the mushroom body calyx of the honeybee

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Social insects possess voluminous mushroom bodies (MBs), higher-order sensory integration centers important for sensory integration, olfactory learning, and memory processes. In the honeybee Apis mellifera, the MBs receive olfactory input in the MB calyx lip region. Within the lip, olfactory projection neurons (PNs) form discrete modular synaptic complexes (microglomeruli, MG) mainly with dendrites from MB intrinsic neurons (Kenyon cells, KCs). In earlier studies using classical immunohistology we demonstrated a remarkable plasticity in olfactory MG densities and numbers associated with development (postembryonic brood care), age, sensory exposure, task allocation within the colony, and olfactory long-term memory formation. To take these analyses to the next level of detail, we applied serial ultrathin section electron microscopy to reveal novel features of olfactory MG at the level of synaptic sites and their connectivity. Young nurse bees and experienced foragers express differences in structure and numbers of presynaptic active zones (AZs) and numbers of postsynaptic partners per AZ. The underlying mechanisms of this synaptic reorganization at individual PN boutons, however, are largely unknown. We currently push the limits of the so far achieved resolution in all three dimensions, and apply 1) the correlative light electron microscopy technique "array tomography" to explore the distribution of synaptic proteins at AZs, and 2) electron tomography based 3D reconstructions to analyze vesicle cargos and the precise architecture of AZs in the MB olfactory lip PN boutons. We hypothesize that reorganization of olfactory MG in the calyx lip adjusts synaptic divergence/convergence ratios between PNs and KCs and plays important roles in age- and experience-related changes in olfactory processing and memory formation.

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P16

Genetic identification and live imaging of gustatory 2nd-order neurons that link sugar detection and feeding/ reward systems in Drosophila

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Sugar stimuli detected by the gustatory system serve as reward cues for associative learning as well as triggers for immediate feeding behaviors. With a relatively simple nervous system and a plethora of genetic tools, Drosophila affords an excellent model for mapping neural circuits involved in these behaviors. Previous work has revealed that gustatory sensory neurons (GSNs) send information about sugar from the mouth to a specific subregion of the brain. Octopaminergic (OA) neurons then mediate the reward by transmitting the signal required for associative learning, while specific brain neurons called the Fdg neurons are required for commanding the feeding behavior sequence. The GSNs have no direct connections to the reward or feeding circuits, suggesting that unidentified neural circuits link these systems.

To clarify these circuits, we conducted anatomical screening to identify gustatory 2nd-order neurons (G2Ns) that receive synaptic inputs from the sugar-sensitive GSNs. Starting from more than 5,000 GAL4 strains, each of which genetically labels a specific subpopulation of neurons, we obtained 15 types of G2Ns (G2N-1 - 15), whose synaptic contacts onto sugar responsive GSNs were visualized by the GRASP (GFP reconstitution across synaptic partners) technique. Further GRASP analyses revealed that four types of G2Ns directly connect to the OA neurons, while another type of neurons bridge GSNs and the Fdg neurons. Now, we are conducting in vivo G-CaMP imaging of these G2Ns while stimulating the fly's mouth with sugar solution. In these experiments we control duration and time-course of tastant concentration reproducibly to compare neuronal responses of GSNs and G2Ns. We will discuss how differently taste information is encoded in the GSNs and G2Ns.

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P17

Towards the identification of pheromone receptors in the desert locust Schistocerca gregaria

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For the desert locust (Schistocera gregaria), pheromones have been reported to be of high relevance regarding reproductive and aggregation behaviors that underlie the formation of giant swarms. Yet, little is known about pheromone detection in locusts and other hemimetabolan insects. In holometabolan insects (such as flies and moths), pheromone detection is mediated by olfactory sensory neurons (OSNs) on the antennae that express pheromone receptors (PRs) belonging to the superfamily of odorant receptors (ORs). The pheromone-reactive OSNs are endowed with a characteristic marker protein termed SNMP1 that is supposed to function as co-receptor transferring pheromones to adjacent PR proteins.

To unravel olfactory receptors involved in locust pheromone detection, we elucidated the OR repertoire of Schistocerca gregaria by analyzing an antennal transcriptome, leading to the identification of ~120 OR types. In search for putative PRs, a larger number of these ORs were tested for possible co-expression with SNMP1. These approaches revealed that in a small subgroup of the ORs, designated as b-OR group, most members were co-expressed with SNMP1 in antennal OSNs. For almost all b-ORs from Schistocerca gregaria, orthologous sequences were found by database analyses in the related species Locusta migratoria; a scenario reminiscent of orthologous PRs previously identified in related moth species. Based on these observations, we consider b-ORs as candidate PRs.

To evaluate a potential involvement of b-ORs in pheromone detection, their responsiveness to the pheromonal compounds phenylacetonitrile and acetophenone from Schistocerca gregaria was assessed in "DREAM" (deorphanization of receptors based on alteration of mRNA concentration) experiments. These approaches indicated that some b-ORs were activated by either phenylacetonitrile or acetophenone. Currently, heterologous expression systems are used to further scrutinize responsiveness of b-ORs to locust pheromones.

P18

Expression of SNMP1 and candidate pheromone receptors in palps of the mouthparts from the desert locust Schistocerca gregaria

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In its gregarious phase, following massive reproduction and aggregation, the desert locust (Schistocerca gregaria) is capable of forming huge swarms that tremendously endanger crop yields in the invaded regions. In locusts, reproductive behavior and aggregation have been reported to be controlled by pheromones. Detection of pheromones in insects is mediated by specialized olfactory sensory neurons (OSNs) on the antennae that are endowed with pheromone receptors (PRs) and characterized by the expression of the "sensory neuron membrane protein 1" (SNMP1). Therefore, SNMP1 is considered as a molecular marker for pheromone- responsive OSNs. Unexpectedly, in Schistocerca gregaria, PCR approaches showed that SNMP1 expression was not confined to the antennae but also occurred in the labial and maxillary palps of the mouthparts. In situ-hybridizations demonstrated SNMP1 expression in a number of cells in the tip region of palps and adjacent to so-called "terminal sensilla" that are partially olfactory. In this regard, two-color in situ-hybridizations revealed that SNMP1 expression in palps was only detectable in a subset of cells positive for the odorant receptor co-receptor (ORCO), indicating that SNMP1 was expressed by OSNs. To further scrutinize a potential involvement of SNMP1-positive OSNs of palps in pheromone detection, the expression of recently identified putative PRs from Schistocerca gregaria in these cells was tested. It was found that similar to the expression of these candidate PRs in SNMP1-positive antennal OSNs, some of these receptors were expressed in subpopulations of SNMP1-positive OSNs from palps.

Our findings suggest that OSNs of palps expressing SNMP1 and putative PRs might contribute to pheromone detection. Since gregarious locusts huddle and their palps are considered to mediate contact chemosensation, relevant locust pheromones could be transferred and detected during direct physical contact.

P19

A neural-network model to investigate peripheral olfactory evolution during dietary specialization

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Numerous studies have shown how specific animals' suites of chemoreceptors are tuned to their dietary preferences.

However, despite the prevalence of dietary specialization, we know surprisingly little about general rules for olfactory evolution associated with these dietary shifts. Here, we propose a biologically realistic neural-network model to explore peripheral olfactory evolution during dietary specialization, assuming negligible evolution in the central nervous system. The model is informed by our detailed knowledge of the organization of dipteran olfactory systems: receptors vary in promiscuity, the number of receptors is far smaller than the number of detectable compounds, and the valence of a receptor is fixed. By assigning the network different olfactory tasks—setting some stimuli as appetitive and others as aversive—and examining the effect of training on weights in the network, we investigate how olfactory-receptor tuning and number are expected to evolve during dietary specialization. This approach can also be used to identify candidate compounds important in specific olfactory tasks, such as host seeking by mosquitoes. Domestic Aedes aegypti mosquitoes strongly prefer human odour over that of other animals, while the closely related forest form is more ambivalent. By training the neural network to have these same preferences, we can identify compounds that may be particularly relevant to these mosquitoes. We will present preliminary results based on body-odour data from a small set of animals as well as plans for future work to sample from a much larger panel of animals. Although this work is in its early stages, we hope it will provide insight into both general rules for olfactory-receptor evolution and the specific evolutionary changes that underlie preference for humans in a major disease vector.

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P20

Interaction of food odors and sex pheromone in Drosophila melanogaster

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In Drosophila melanogaster, the males produce a sex pheromone, cis-vaccenyl acetate (cVA) that elicits gender-specific behavior in both males and females. Our study demonstrates that exposure to the male-produced sex pheromone cVA in combination with the food odor vinegar evokes an enhanced and synergistic functional response in the primary olfactory center of virgin female flies. In our study, we elucidated the neuronal mechanism underlying this synergistic response at an anatomical, functional and behavioral level. This effect arises within the neuronal network in glomerulus DA1, in the antennal lobe and is mediated by electrical synapses. The synergistic response in virgin females leads to an increased sensitivity to the sex pheromone and therefore an enhanced female receptivity during courtship. This mechanism is highly useful, since it promotes mating in females when food is present, i.e. when the nutritional supply of the female and its offspring is guaranteed. Altogether, our results suggest that lateral excitation via gap junctions modulates odor tuning in the antennal lobe and drives synergistic interactions between two ecologically relevant odors, representing food and sex.

THEME II – VERTEBRATE TASTE AND OLFACTION: PERIPHERY

P21

Predicting odor similarity of complex mixtures from molecular approach

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Olfactory research was and is still challenged by predicting odor characteristics of odorants from their molecular structure. Different teams have been working on establishing successful predictive approaches, but many of them apply to single odorants. However, odors we perceive in every-day life are mixtures including many different odorants at varying concentrations. The odor quality of such mixtures can be perceived as elemental (components odors can be perceived within the mixture) or configural (components odors blend into a new odor perceived as an entity). We applied the angle distance model, developed by Snitz et al (2013), which successfully predicted the similarity of complex mixtures composed of iso-intense components using molecular structure. We applied the model to elemental and configural mixtures made of 6 odorants, their sub-mixtures and odorants alone. We also extended the model to take into account the intensity of each odorant in mixtures because odorants were not intensity-balanced. To do so, we calculated the ratio of each component in the mixtures based on their relative concentration. Perceptual similarity was rated for 63 pair comparisons by a panel of 60 subjects (Romagny et al, 2017). The angle distance model well predicted perceptual similarity ($r=0.64$, $p<0.001$) in our benchmark dataset that includes mixtures with different levels of complexity, using component odors with various intensities, and eliciting both elemental and configural percepts. However, the extended model succeeded better at the predictive task ($r=0.71$, $p<0.001$). Thus, taking into account intensity along with structural parameters

improved not only the predicted similarity among mixtures of odorants but also allowed to determine which mixtures were processed in a more elemental and configural way by the olfactory system. This finding should lead to better results in predictive approaches based on molecular structures.

P22

Bitter and sweet compounds activate a subset of rat tracheal epithelial cells

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Bitter and sweet receptors (T2Rs and T1Rs) are expressed in many extra-oral tissues including the epithelium of different portions of airways system. Our previous data show the localization of the T1R3 sweet receptor both on the cilia of ciliated cells and in isolated chemosensory cells of rat trachea. Moreover, the same cells express also different components of taste transduction pathway such as PLCβ2 and α-gustducin. Therefore, we performed confocal Ca^{2+} imaging recordings on acute tracheal slices model to investigate if bitter and sweet substances could activate physiological responses in tracheal epithelial cells. We stimulated the cells with denatonium benzoate, a T2R agonist, and with several artificial sweeteners including sucralose, saccharin and acesulfame-K. We found that about 30 % of the cells responded to bitter stimulation with denatonium benzoate. Moreover, artificial sweeteners activated different cell percentage ranging from about 5% for sucralose to 27% for saccharin. Pharmacological experiments showed that both denatonium and artificial sweeteners induced a PLC-mediated release of Ca^{2+} from internal stores. We also investigated if agonists of T2R bitter receptors and artificial sweeteners were able to activate different or overlapping populations of cells: results showed that bitter and sweet substances activated a partially overlapping subpopulation of tracheal epithelial cells. Our results provide new evidence that a subset of rat tracheal epithelial cells is chemosensor, capable to detect both bitter and sweet compounds.

P23

Alterations of olfactory epithelium in stomatin knock-out mice

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Protein of the stomatin family are characterized by the presence of structurally conserved core domain called stomatin-domain of about 120 residues. In mammal genome, 5 members have been identified: stomatin, STOML-1, STOML-2 and STOML-3 and podocin. Several studies began to reveal some common aspect of the physiology of stomatin proteins, in particular they mostly localized to membrane domains forming oligomers and they can modulated ion channel activity, even if the precise mechanism of this regulation is still unclear. Some previous data also shown that some members of stomatin protein family are expressed in the olfactory epithelium. Here using RT-PCR we confirm that stomatin, STOML-1, STOML-2 and STOML-3 are expressed in mouse olfactory epithelium. Moreover, by immunohistochemistry and X-gal staining we found that stomatin are localized in mature olfactory sensory neurons (OSNs). Then we started to characterize the phenotype of two mouse lines, a single knock out for STOML-3 and a triple knock out for stomatin, STOML-1 and STOML-3. We found that in the triple KO there is a significant reduction of the number of mature olfactory sensory neurons especially in in the posterior part of the epithelium. However, no significant defects are evident in the morphology of the olfactory sensory neurons and in the expression of component of olfactory transduction cascade such as CNGA2 and TMEM16B. Finally, we used the electro-olfactogram (EOG) recording to investigate the physiological response to odors in our knock out models. While the STOML-3 single knock out did not show significant difference in odor response respect to wild type mice, in triple knock out the response to odorant in posterior part of olfactory epithelium is significantly reduced correlating with the observed reduction of number of OSNs. Additional studies are necessary to fully characterize the physiological role of stomatin protein in olfactory epithelium.

P24

Receptomics: Calcium imaging of sensory receptor cell arrays in a microfluidic system and novel applications for food screening

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Reverse-transfected cell arrays in microfluidic systems have strong potential to perform large-scale parallel screening of

GPCR libraries. We have successfully combined the multivariable nature of a receptor cell array with microfluidics allowing for controlled and sequential sample dosing. Our receptomics platform uniquely allows for dosing of small sample quantities against a large receptor library including controls for host cell responses and sample colour. Compared to existing microtiterplate systems it can be applied more efficiently for the screening of off-target effects and the discovery of bioactivities in complex extracts. The sequential injection format allowed the development of powerful spot-based statistical models to discriminate between a host cell response and the superimposed specific GPCR response.

The receptomics method involves reverse transfection of HEK293 cells, imaging by stereo-fluorescence microscopy in a flowcell format, real-time monitoring of cytosolic Ca_{2+} fluctuations, and automated statistical analysis of GPCR responses to sequential sample exposures.

By varying the GPCR DNA concentration in reverse transfection, the sensitivity and robustness of the receptor response for sequential sample exposures was optimized. We show a series 14 sequential sample injections with specific and reproducible response patterns for the entire series. We show proof of principle results using arrays with a set of bitter taste receptors and complex extracts like tomato juice and coffee and demonstrate the effectiveness of the methods to handle matrix interference and host cell response.

These findings provide a confident outlook on introducing this system as a novel high throughput GPCR screening platform with important applications in the area of sensory research.

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P25

Effect of dipeptides on salty taste in rat taste papillae cells

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Saltiness is one of the five basic tastes often elicited by NaCl. It is essential to reduce sodium in our diet because excessive sodium intake can cause many health problems. Previous human sensory studies showed that some dipeptides including arginine-glycine (RG), arginine-serine (RS), arginine-valine (RV) and arginine-methionine (RM) from fermented food could enhance salty taste, but the mechanism is still not fully understood. As a result, our study aims to evaluate the effect of these arginyl dipeptides on salty taste in rat taste papillae cells and to investigate the mechanisms involved. The methodologies we used included immunocytochemistry, RT-PCR, western blotting and confocal calcium imaging. Results showed that isolated rat circumvallate (RCV) and rat foliate (RFL) taste papillae cells expressed types I, II, III taste cell markers and receptors for five basic tastes. These cells also responded to stimuli of five basic tastes. The arginyl dipeptides elicited responses of the RCV taste papillae cells in a dose-dependent manner. When evaluating the function of 10, 50 and 100 μ M dipeptides on salty taste in RCV taste papillae cells, we found that 10 μM RS enhanced the 150 mM NaCl-elicited responses significantly ($p<0.05$) while RV showed no significant enhancement. In summary, our preliminary results indicated that 10 μ M RS may enhance the salty taste through amiloride-insensitive mechanisms since RCV taste papillae cells had no functional amiloride-sensitive salty taste receptors.

P26

Stimulation of chemosensory brush cells triggers cholinergic contraction in the mouse gall bladder

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[Objective] Cholinergic chemosensory (brush) cells are considered to monitor the chemical composition of the lining fluid at mucosal surfaces through the bitter taste transduction cascade. Upon stimulation, they trigger avoidance reflexes. Such cells are particularly frequent in the gall bladder. We here set out to determine mechanisms that evoke acetylcholine (ACh) release from brush cells, and what potential effects this might have in the gall bladder.

[Methods] Isolated mouse bladder contraction was studied in organ bath recordings. Stimuli were dextromethorphan and, in appropriate strains expressing channelrhodopsin (ChR2) either in cholinergic nerve fibres or in chemosensory cells, LED stimulation (optogenetics). ACh release was quantified by HPLC. Intracellular calcium concentration ($[Ca^{2+}]_i$) was recorded in isolated cells.

[Results] Muscarine (100 μM) evoked gall bladder contraction, reaching about 60% of that resulting from stimulation with cholecystokinin (0.1 μM). Dextromethorphan (100 μM), a bitter tastant, caused $[Ca^{2+}]_i$ increase in 4/10 brush cells and caused gall bladder contraction. Fifty percent of this contraction was cholinergic (atropine sensitive) (n=8, p=0.02). This cholinergic component required the taste transduction cascade (abolished in TRPM5 $^{-/-}$ mice, n=4) and brush cells (abolished in Pou2f3 $^{-/-}$ mice, n=4). Signaling to nerve fibers was not involved (no effect of TTX, 1 μM, and A-803467, 5 μM). Optogenetic stimulation of explanted gall bladders from ChAT-ChR2(H134R)-EYFP mice (ChR2 expression restricted to brush cells), but not from control strains, resulted in an increase in ACh content (from 1.74±0.38 to 10.20±2.47 nM; n=10, p=0.006) in the medium and evoked atropine-sensitive (n=8, p=0.04) bladder contraction.

[Conclusion] Depolarization triggers ACh release from gall bladder brush cells, and smooth muscle contraction is one of the effector mechanisms triggered by cholinergic brush cell stimulation.

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P27

Bitter taste receptors and components of the taste transduction cascade in the mouse gall bladder

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[Objectives] Taste-sensing type 2 (bitter) receptors (Tas2R) are widely expressed in extra-oral tissues. In chemosensory (brush) cells of various epithelia they are linked to the canonical taste transduction cascade (CTTC), and serve to detect hazardous compounds at the mucosal surface. Airway and urinary bladder smooth muscle relax in response to bitter agonists through a still ill-defined pathway. Previously, we found that the mouse gall bladder epithelium harbours many brush cells. We now determined the repertoire and cellular distribution of Tas2R and the CTTC in the mouse gall bladder, and their impact on smooth muscle tone.

[Methods] Gall bladder Tas2R expression patterns were assessed by RT-PCR and with in situ hybridization (ISH). ISH and immunohistochemistry were utilized to show the localization of the CTTC components. Gall bladder contraction was studied in organ bath recordings in wildtype, TRPM5$^{-/-}$, and Tas2R triple-knockout mice (Tas2R143/135/126$^{-/-}$). Intracellular calcium concentration ($[Ca^{2+}]_i$) was recorded in isolated cells.

[Results] mRNAs for several Tas2R, including members 108, 126, 135, 137, 138, and 143, were present in the gall bladder, and localized to solitary cells in the epithelium. Expression of CTTC members, including α-gustducin, PLCβ2, and TRPM5, was detected in cholinergic brush cells. Bitter tastants (denatonium, quinine, noscapine) dose-dependently relaxed pre-contracted gall bladder in wildtype, TRPM5$^{-/-}$ and Tas2R143/135/126$^{-/-}$ mice. Quinine and denatonium caused an increase in $[Ca^{2+}]_i$ in isolated smooth muscle cells.

[Conclusions] Both, bitter receptors and the taste transduction cascade are concentrated in chemosensory brush cells in the mouse gall bladder. The direct relaxant effect of various bitter agonists on gall bladder smooth muscle most likely operates through an independent pathway.

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P28

In silico modelling and evolutionary relatedness of the OR37 subfamily of olfactory receptors.

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The OR37 subfamily of odorant receptors(ORs) represents a rather ancient gene cluster which exists exclusively in mammals. In mouse the OR37 main olfactory receptors A, B and C activated by the long-chain aliphatic aldehydes pentadecanal, hexadecanal and heptadecanal, respectively project via the main olfactory bulb to directly synapse on arginine vasopressin neurons in the paraventricular nucleus of the hypothalamus (PVN). Exposure to these aldehydes has been shown to inhibit hypothalamic corticotropin releasing hormone neuron activity and possibly the glucocorticoid stress response suggesting a role in mediating a phenomenon called social buffering. The OR37 olfactory receptor family that mediates the effects of the OR37 ligands is unusually conserved across species, suggesting there is the potential to open a new area of research in identifying similar ligand mixtures that are effective for other mammalian species.

Firstly, we identified the OR37 gene family repertoire in 13 species of placental mammals for which deep-coverage

genome sequences are available. Those placentals with OR37A, B and C orthologs are candidates for ligand screening. Here we report for the first time the building of a novel 3-D mOR37 protein structure which we will use to develop an in silico screening model for characterization of potential ligands that are predicted to functionally activate OR37 receptors in other mammalian species. This has potential ethical and productivity impacts for food production, the welfare of companion animal and captive species, as well as numerous potential applications in human and veterinary medicine.

P29

Optical control of mouse Trpc2 and human TRPC6 channels using photoswitchable diacylglycerols

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The Trpc2 cation channel is a central transduction element in sensory neurons of the mouse vomeronasal organ (VNO). The finding that Trpc2 is also expressed in sensory neurons of the main olfactory epithelium (MOE) where it is required in type B cells for the detection of low environmental oxygen has sparked renewed interest in its function. The second messenger signaling mechanisms underlying activation of Trpc2 and its corresponding cellular responses are still debated. We assessed and established the application of a unique family of light-sensitive, photoswitchable diacylglycerols (DAGs), termed PhoDAGs, in TRP channel research. PhoDAGs can be used to rapidly activate and deactivate DAG-sensitive TRP channels in living cells including native Trpc2 channels in chemoreceptive neurons of the mouse olfactory system and human TRPC6 channels expressed in HEK cells. We developed an approach for combined PhoDAG photoconversion and Ca^{2+} imaging based on confocal laser scanning microscopy that can even be employed in acute tissue slices, thus enabling both large-scale mapping of DAG-evoked neuronal activation and localized stimulation and mapping in small cellular compartments that otherwise would not be accessible. This approach reveals the existence of comparatively slow DAG-activated Ca^{2+} transients in a recently discovered oxygen sensor of the mouse olfactory epithelium (known as type B cells) and in the related type A cells. This toolset provides a unique experimental platform that should enable us to better understand the gating mechanisms of DAG-sensitive TRP channels. We are currently expanding this approach to neurons of the central nervous system that express other TRP channel isoforms. Supported by Deutsche Forschungsgemeinschaft grants SFB-Transregio 152 and INST 256/427-1 FUGB, and the Volkswagen Foundation.

P30

Cat chemical cues effects on mouse estrous cycle depend on concentration and presentation mode

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Unique amino acid L-felinine and its volatile derivatives may be used by the house mouse to recognize potential predators, their physiological status and may affect reproductive output in mice. The aim of the current study: to examine the role of concentration and different exposure modes of L-felinine in regulation of estrus cycle in the house mouse. We selected three concentrations of L-felinine comparable with naturally occurring in the urine of adult male and female cat (0,05%, 0,1%), or smaller female or immature cats (0,025%). The following exposure modes were used: permanent, regular and spontaneous. Different modes of exposure simulate predator appearance in various proximity to the prey habitat as well as the different duration and regularity of the probable contact. We recorded: the length of the estrous cycle, the number of ovulations, the latency of the first estrous after exposure, number of omitted cycles. To measure fecal estradiol we used an ELISA technique (Kvasha et al., 2018). To monitor estrous cycle phase we calculated fecal estradiol baseline for each animal individually; concentrations above the baseline were considered as a beginning of luteal phase. We observed significant effect of both presentation mode and the concentration of L-felinine on parameters of the estrous cycle in the house mouse (Two-way ANOVA, $F(3, 52) = 3.3057$, $p = 0.0272$, $n = 38$; concentration of felinine $F(3, 52) = 3.1615$, $p = 0.03216$, $n = 38$). . The most profound effect was found in group under regular exposure to L-felinine (0.05%) ($p = 0.0000$, $n = 45$, $\chi 2$). A regular mode of exposure to L-felinine (0.05%) models the situation when mice are at the border of the adult cat's average daily ranging habitat (3.41 ha, on average), which is defined as the mean maximum area range (Thomas et al., 2014). We did not observe habituation to repeated presentations of the predator signals, which indicates the innate nature of the response.

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P31

Solitary chemosensory cells (SCC) release acetylcholine upon stimulation by a formylated bacterial signal peptide to boost mucociliary clearance

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We previously showed that a specific formylated bacterial signal peptide (FL185), produced by various pathogens, e.g. E. coli and Salmonella typhimurium, increases mucociliary clearance in the murine trachea. This depends on the canoncial taste transduction cascade (TRPM5 and PLC β_2). Atropine sensitivity gave evidence for cholinergic signaling. We here examine whether solitary chemosensory cells (SCC) are the key cell type for recognition of this formyl peptide in the trachea.

Particle transport speed (PTS) was studied in C57Bl6 mice, in mice lacking components of the bitter taste transduction cascade (ITPR3$^{-/-}$ and Tas2r143/135/126 triple KO-mice) and in mice lacking SCC (TRPM5-DTA and Pou2f3$^{-/-}$). ACh was measured by HPLC.

ACh was increased in supernatants of tracheas after stimulation with FL185 (10 µM) (70 ±17 to 158 ±36 nM; p=0.02; n=6; paired t-test). This was not the case in TRPM5 $^{-/-}$ mice (112 ±70 to 60 ±20 nM; p=0.20; n=3). FL185 increased PTS from 44 ±2 to 75±3 µm/s (mean±SEM; p <0.0001; n=23; paired t-test). Atropine (1 µM), a general muscarinic receptor antagonist, reduced the effect by 75 % (p=0.0025; n=7, unpaired t-test). 4 -DAMP (1 µM), a specific M3 receptor antagonist, reduced it by 84 % (p=0.003; n=6). Neither the nicotinic antagonist mecamylamine (p=0.38; n=5) nor TTX (p=0.52; n=5) changed the increase of PTS by FL185. In Tas2r143/135/126$^{-/-}$-mice, the effect was reduced by 32% (p=0.048; n=12). In ITPR3$^{-/-}$-mice, the effect was abolished (p=0.75; n=7; paired t-test). In mice lacking SCC, Pou2f3$^{-/-}$ (p=0.15; n=4) and TRPM5-DTA mice (p=0.17; n=5), the effect was abolished.

The cell ablation experiments clearly demonstrate that the perception of a specific pathogen associated formyl peptide crucially depends on the presence of SCC in the trachea. The underlying signal mechanism involves elements of the bitter taste transduction cascade and triggers stimulation of mucociliary clearance by release of ACh acting upon muscarinic (M3) receptors.

P32

Bitter taste signals are modulated by calcium-sensing receptor through the interaction with T2Rs

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Receptors for each basic taste are thought to be expressed in separate populations of taste bud cells. For example, bitter taste is elicited by activation of T2R bitter taste receptors. In addition, humans possess orosensory mechanisms to detect compounds that do not elicit a basic taste. We recently demonstrated that the orally administered modulators of calcium-sensing receptor (CaSR) affect orosensory perception. For instance, in human sensory studies, γ-EVG enhances the intensities of umami, sweet, and salty tastes in a CaSR-dependent manner. These phenomena are called 'kokumi' flavor. Importantly, kokumi substances do not elicit any taste itself. Apparently they exert their action mainly via a subset of T2Rs-expressing bitter taste cells which co-express CaSR. To reveal the functional role of CaSR in T2Rs-expressing taste cells, we investigated the functionality of cells co-expressing CaSR and T2Rs in the heterologous HEK cell system. We found physical interaction between CaSR and T2Rs. In co-expressing cells, CaSR modulated bitter substances-induced cellular responses, whereas T2Rs did not affect γ-EVG-induced cellular responses. Furthermore, the bitter substance-induced cellular responses in co-transfected cells were attenuated by inactivating CaSR using its antagonist NPS2143 or Ca2+ chelation with EDTA. Additionally, the co-expressed cells responded faster to stimulation with bitter substances

compared with cells expressing T2Rs alone. These response patterns are similar to that of CaSR-expressing cells stimulated by γ-EVG. Further, in human sensory test, application of CaCl2 at low concentration, which is tasteless, significantly reduced perceived bitterness of quinine. Taken together, these results indicate that activation state of CaSR affects T2R activation by bitter substances through CaSR-T2R interaction and modulates bitter taste signaling. Our results strongly suggest that CaSR acts in bitter-sensing cells to weaken bitter taste perception.

P33

Developing gene therapy approaches for anosmia induced by CNG ahannel mutations

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Ion channels are critical for regulating excitability in many cell types including olfactory sensory neurons (OSNs). In OSNs ion channels are responsible for depolarizing the cell in response to odor stimulation, initializing an action potential and synaptic transmission. Channelopathies are a class of human genetic disorders in which ion channel function is disrupted leading to defects in multiple organ systems. Disruptions in ion channels are known to cause epilepsy, cardiac arrhythmias, blindness, deafness, alterations in pain sensitivity, and anosmia. Deletions of several different ion channels in mouse models also cause anosmia, indicating that olfactory signaling can be affected at multiple steps. Additionally, mutations in several ion channel subunits, including those that comprise the olfactory cyclic nucleotide gated (CNG) channel and sodium channels, have been found in patients with anosmia and hyposmia. Gene therapy approaches offer the ability to restore functional copies of mutated genes and correct defects. Previous work has shown this to be possible in the olfactory system. The goal of this study is to test the ability of gene therapy to correct defects in olfactory function of two mouse strains with targeted deletions in CNG channel subunits. CNGA2 and CNGB1b are critical subunits of the olfactory CNG channel necessary for odor detection and their loss leads to anosmia. Using adenovirus vectors, we delivered functional copies of the missing genes to mutant OSNs to test the ability to restore olfactory function. En face imaging reveals correct localization and robust expression of the ectopic subunits. Immunohistochemistry staining for activity markers, such as S100a5 and tyrosine hydroxylase are used to determine if expression restores neuronal function. The results from the proposed research will be important for helping to develop therapies for patients with anosmia due to channelopathies.

P34

Odorant receptors containing conserved amino acid sequences in transmembrane domain 7 display distinct expression patterns in mammalian tissues

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Mammalian genomes are well established, and highly conserved regions within odorant receptors that are unique from other G-protein coupled receptors have been identified. Numerous functional studies have focused on specific conserved amino acids motifs, however, not all conserved motifs have been sufficiently characterized. Here, we identified a highly conserved 18 amino acid sequence motif within transmembrane domain seven (CAS-TM7) which was identified by aligning odorant receptor sequences. Next, we investigated the expression pattern and distribution of this conserved amino acid motif among a broad range of odorant receptors. To examine the localization of odorant receptor proteins, we used a sequence-specific peptide antibody against CAS-TM7 which is specific to odorant receptors across species. The specificity of this peptide antibody in recognizing odorant receptors has been confirmed in a heterologous in vitro system and a rat-based in vivo system. The CAS-TM7 odorant receptors localized with distinct patterns at each region of the olfactory epithelium; septum, endoturbinate and ectoturbinate. To our great interests, we found that the CAS-TM7 odorant receptors are primarily localized to the dorsal region of the olfactory bulb, coinciding with olfactory epithelium based patterns. Also, these odorant receptors were ectopically expressed in the various non-olfactory tissues in an evolutionary constrained manner between human and rats. This study has characterized the expression patterns of odorant receptors containing particular amino acid motif in transmembrane domain 7, and which led to an intriguing possibility that the conserved motif of odorant receptors can play critical roles in other physiological functions as well as olfaction.

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P35

Odor habituation can be represented by early olfactory event related potential

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Despite involvement of central nervous system(CNS) in odor habituation, previous studies in N1 of event related potential(ERP) showed no changes in odor habituation not like other sensory systems. N1 signal is known as represent processing of exogenous stimulus and decreased in habituation. According to previous odor habituation studies, N1 was regarded as negative potential near 200-600ms. However, several studies showed that odor signal in CNS processed earlier than 200ms. For these reasons, we studied whether early N1 signal of olfactory ERP may be changed in odor habituation. To verify the change of early N1 signal in odor habituation, we performed an odor habituation behavior test and EEG experiments. There were three different conditions: None (Distil water offered in first step), Different (two different odors offered in first and second step) and Same (a same odor offered in first and second step). We found channels that showed significantly different N1 amplitude across the conditions and correlated with the behavior test within 200ms. Furthermore, topographical distribution of early N1 amplitude was different depending on brain areas. These results suggest that early N1 signal of brain may be involved in odor habituation and odor habituation was processed differently depending on brain areas. Our studies suggest that odor habituation may be represented by the change of N1 in the brain and these processes may be different depending on brain areas.

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P36

Wnt/β-catenin Signaling Affects Two Modes of Neurogenesis in the Zebrafish Olfactory Epithelium

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Olfactory sensory neurons (OSNs) have a limited life span and need to be generated constantly by maintenance neurogenesis to prevent olfactory loss at old age. In addition, the olfactory epithelium (OE) is capable of mounting efficient regenerative responses to acute tissue damage. In zebrafish, non-identical progenitor pools with distinct tissue distribution contribute to OSN maintenance and OE repair, however, the signals that regulate these two modes of OSN neurogenesis are not well characterized.

We have used gene expression profiling by RNA sequencing to identify molecular signaling pathways that are significantly upregulated in an experimental model of OE regeneration. We find that components of the canonical Wnt/β-catenin signaling pathway are strongly activated early after damage to the OE. In the intact OE, β-catenin-positive cells are restricted to regions of maintenance neurogenesis at the central and peripheral edge of the sensory tissue, while repair neurogenesis is induced in the sensory OE upon damage.

To test the contribution to Wnt/β-catenin signaling to these two modes of OSN neurogenesis functionally, we manipulated Wnt activity pharmacologically in the intact and lesioned OE. Activation of the Wnt pathway (LiCl, CAS 853220-52-7) promoted strong cell proliferation responses, including responses in the sensory OE that resembled the pattern of neurogenesis under damage conditions. Inhibitors of the pathway (ICRT-14, IWR), on the other hand, suppressed, but did not abolish, maintenance neurogenesis and had a suppressive effect on damage-induced proliferation in the sensory OE. Our results suggest that Wnt/β-catenin signaling is necessary and sufficient to induce cell proliferation from two types of neuronal progenitor populations in the OE that selectively contribute to maintenance and repair neurogenesis.

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P37

Purinergic signaling promotes neurogenesis in the zebrafish olfactory epithelium

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The peripheral olfactory epithelium (OE) is unprecedented in its capacity to generate new olfactory sensory neurons (OSNs) throughout life and to regenerate efficiently from acute injury. OSN neurogenesis must be tightly controlled as over- or underproduction of nerve cells would be detrimental to olfactory function, however, the underlying signals are only poorly characterized. Here we examine the

contribution of purinergic signaling to OSN neurogenesis from basal progenitors in zebrafish, using Ca^{2+} imaging of purinergic responses, molecular identification of purine-sensitive cell types, and cell proliferation assays after purine stimulation.

To identify non-neuronal cells that may be implicated in OSN neurogenesis, we studied physiological responses to a series of related purine compounds by measuring Ca^{2+} release on vibratome sections through the OE. While ATP induced responses in the basal and apical OE, MeSATP response where restricted to a subset of basally located cells. Moreover, apical and basal responses were blocked differentially by the P2Y receptor antagonist Suramin. To identify these cells, we performed morphometric comparisons of purine-responsive cells and cell populations expressing cell type-specific molecular markers. Most ATP-sensitive cells showed overlap with sox2 expression, which could be further subdivided into cytokeratin II-positive sustentacular cells (SCs) and a more basal krt5-positive cell population, which resembled horizontal basal cells.

To test the biological relevance of purinergic signaling in the OE more directly, we stimulated fish by intraperitoneal injection of ATP and observed an increase in neurogenic cell proliferation in the OE, which was revertable by Suramin treatment. The results suggest that purine release in the OE, eventually from damaged or dying OSNs, stimulates OSN neurogenesis from purine-sensitive basal progenitor cells.

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P38

Morphological features of the uropygial gland of the jungle crow Corvus macrorhynchos

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Recently, the avian uropygial gland has attracted interest in regard to the functional significance of its secreted volatile compounds in avian social communications. In passerines, the weight of the uropygial gland is a maximum during the reproductive season. The passerine uropygial gland appears to be an appropriate subject for histological analysis to examine the relationship between social communications and uropygial secretions. Hence, the purpose of this study was to clarify differences in the uropygial gland of jungle crows according to development and season. A total of 32 jungle crows (Corvus macrorhynchos; males: n = 17, females: n = 15) were used in this study. Captured birds were identified according to color of the upper palate and were divided into three age groups (<1 year, 1–2 years, >2 years). The uropygial gland was embedded in paraffin and sectioned into 3- or 5-μm slices using a sliding microtome. Sections were stained with hematoxylin and eosin, Elastica van Gieson, and a proliferating cell nuclear antigen (PCNA) antibody. Microscopic evaluation revealed that the uropygial gland was of compound tubular gland consisting of several secretory tubes. The glandular cavity differed from that seen in other birds, and was covered with mesh-patterned tissue composed of collagen fibers. The ventral area of the uropygial papilla was developed in older birds but not in the <1- and 1–2-year-old birds. PCNA antibody staining was concentrated in the basal layer of secretory tubes. In addition, PCNA positive cells formed elliptic clusters in the mesh-patterned tissues. Elliptic clusters were observed in birds that were >2 years old and captured during the reproductive season. These results indicate that the jungle crow's uropygial gland can change with development and season. In addition, the elliptic clusters detected may be related to the composition of volatile compounds that elicit social behavior.

P39

Lipocalin genes expressed in distinct patterns in mouse nasal glands

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In order to be detectable by the olfactory sensory neurons in the nasal cavity of land-living vertebrates, volatile hydrophobic odorants have to traverse an aqueous barrier formed by the mucus layer covering the sensory epithelium. It is believed that the transfer of odorants to their targets is facilitated by odorant binding proteins (OBP) which belong to the lipocalin protein family. The known repertoire of OBPs in mammalian species is rather low in comparison to the huge number and structural diversity of odorous compounds that they can detect. To address the question whether additional members from the lipocalin family might be candidate OBPs, we analyzed three clusters of lipocalin genes for their expression in the mouse nasal cavity. By RT-PCR studies, eleven genes were found to be expressed in the mouse nasal cavity. In situ hybridization experiments revealed that all of them were expressed in the typical OBP-producing glands in the anterior region of the nasal cavity; none of them was detectable in the Bowman's glands below the olfactory epithelium. Double fluorescence hybridizations demonstrated that different subcompartments of the anterior glands express distinct combinations of these lipocalin genes. The data indicate that the repertoire of OBPs might be larger than previously thought and that different glands produce distinct mixtures of these proteins.

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P40

N-geranyl cyclopropyl-carboxamide, an umami taste modulator interrupts salicin binding to hTAS2R16 bitter taste receptor

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We recently demonstrated that umami peptides effectively suppress salicin-induced intracellular Ca^{2+}-influx in cells expressing hTAS2R16 bitter taste receptor and a subsequent point mutation analysis provides evidence that umami peptides directly bind to N96 and P44 allosteric sites in hTAS2R16. From our previous research in human and animal model, we found that N-geranyl cyclopropylcarboxamide (NGCC), a novel synthetic compound, acts as an umami modulator interacting with a Ca^{2+}-dependent transduction pathway. Herein, we investigated the interactions between NGCC and salicin-induced bitter taste in cells expressing hTAS2R16. NGCC selectively suppressed the salicin-induced intracellular calcium influx in a time-dependent manner (IC_{50} = 104.1±1.14 μM). The efficacy of NGCC suppressing salicin-induced activation of hTAS2R16 was stronger than that of umami active dipeptides, Glu-Glu (IC_{50} = 9.67±1.22 mM), Glu-Ser (IC_{50} = 8.68±1.25 mM), Glu-Asp (IC_{50} = 16.63±1.32 mM), or Asp-Asp (IC_{50} = 15.85±1.40 mM). Based on our previous point mutation analysis for umami peptides, two known probenecid-insensitive mutants hTAS2R16 N96T and P44T were thus selected for experimental testing. Like probenecid and umami peptides, NGCC-induced suppression of intracellular Ca^{2+} response induced by salicin in wild type hTAS2R16 was significantly reversed in the N96T and P44T mutants. These data support to the suggestion that bitter taste receptor, at least hTAS2R16, has a binding pocket for wide range of umami substances not just umami peptides. This research was supported by National Research Foundation of Korea (NRF) grant NRF-2017R1A2B2008527.

P41

Spheroid culture of rat olfactory receptor neurons and its application for bioelectronic nose

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A novel concept of cell-based biosensor mimicking odorants encoding process of the mammalian ORN was proposed and developed. Rat ORN precursor spheroids were formed by using recombinant protein (REP; TGPG[VGRGD(VGVPG)$_6$]$_{20}$WPC) and used as a bioelement and combined with commercialized multi electrode array (MEA). Spheroid culture of ORN precursors realized stable maintenance and long-term storage of ORN precursors with preservation of stemness characters. Physiological characters of the differentiated ORNs from spheroids were verified by monitoring change of intracellular calcium concentration upon odorant mixture stimulation and these characters were well preserved in the long-term cultured ORN spheroids. Lastly, differentiated ORN on the MEA generated electrical signals upon odorants stimulation and these signals were collected as signal patterns and analyzed for detecting and discriminating odorant types and concentrations. Developed ORN-based biosensor showed superior repeatability and reproducibility in the odorant detection and the ORN spheroid culture have potentials to be applied in the development of a bioelectronic nose and high-throughput odorant screening.

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P42

The anti-obesity and anti-diabetic natural compound, cuminaldehyde, activates the transient receptor potential Ankyrin 1 (TRPA1)

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TRP channels are involved in various physiological processes as pain, inflammation, metabolism, swallowing function, gut motility, thermoregulation or adipogenesis, as well as the sensory chemical transduction of spicy ingredients in the oral cavity called chemesthesis. Among TRP channels family, TRPA1 is activated by a broad variety of natural molecules giving rise to the pungent, tingling, irritation and burning experience form their consumption. Various classes of chemicals found in plants or spices are able to induce TRPA1 response. TRPA1 can be activated through a covalent binding by the electrophiles isothiocyanates or thiosulfinates. Isothiocyanates are found in wasabi, mustard, horseradish or capers as examples. Thiosulfinates as diallyl sulfide or diallyl disulfide are found in plants as garlic or

onion for the Allium genus. Unsaturated aldehydes among which recognized flavors, as cinnamaldehyde, have shown to elicit big response of TRPA1.

Beside sensory coding of chemesthesis, TRPA1 has been associated to various physiological mechanisms, as gut motility, inflammation or pain. The potent TRPA1 agonist, cinnamaldehyde, is reported to impact metabolism and exert anti-obesity and anti-hyperglycemic effects1. Recently, it has been reported that cuminaldehyde, a structurally close molecule to cinnamaldehyde, possess anti-obesity and anti-hyperglycemic effect as well 2,3. We speculated that this effect might be due to TRPA1 activation. The results of this study show that indeed cuminaldehyde activates hTRPA1. Additionally two natural agonists of hTRPA1 were identified, p-ansialdehyde and tiglic aldehyde, opening the door to new natural class of ingredients to be evaluated for their impact on metabolism.

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P43

Characterization of GPRC5C in murine main olfactory epithelium

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Orphan G protein coupled receptor (GPCR) research has gained momentum in the last few years owing to the multitude of physiological and pathological roles these receptors play. GPRC5C is an orphan which belongs to Class C of GPCRs along with the metabotropic glutamate receptor, the taste receptors and the pheromone receptors. Phylogenetic analysis revealed the evolutionary conservation of GPRC5C among chordates. Although GPRC5C has been implicated in numerous diseases; precise functions haven't been attributed to it yet. We have previously shown via next generation sequencing, that the gprc5c gene is one of the most highly expressed non-olfactory GPCRs, in olfactory sensory neurons (OSN). In the current study, we demonstrate a distinct, punctate localization of GPRC5C in the dendritic knobs of OSNs via immunostaining. To analyze this localization, we employ freeze-fracture replica immunogold labeling (FRIL) and en-section immunogold labeling of epon embedded ultrathin sections of the olfactory epithelium (OE), followed by transmission electron microscopy (TEM), which reveals an association of GPRC5C with cell junctions between the dendritic knobs and adjoining sustentacular cells. A similar association of GPRC5C with cell junctions in MDCK cells overexpressing the protein can be visualized via FRIL. In order to study the functional significance of GPRC5C in the OE, we currently analyze constitutive GPRC5C knockout ($GPRC5C^{-/-}$)mice. Scanning electron microscopy and TEM analysis of epon embedded ultrathin sections of OE, of age matched, wild type and $GPRC5C^{-/-}$ mice show morphological differences between the two, hinting at an altered functioning of the OE in $GPRC5C^{-/-}$ mice. Furthermore, we are analyzing receptor function in the recombinant expression system.

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Serotonin release by the TRPA1-agonist cinnamaldehyde and structural analogues in differentiated Caco-2 cells

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Activation of the transient receptor potential (TRP) channel TRPA1 by cinnamaldehyde (CA) has been shown to stimulate serotonin release in enterochromaffin QGP-1 cells. However, the impact of cinnamaldehyde on serotonin release in enterocytes is not well understood. In addition, since the neurotransmitter serotonin plays a regulatory role in a large variety of gastrointestinal and metabolic functions, it is of interest to study which structural characteristics determine the serotonin release by enterocytes. Thus, we here analyzed serotonin release in differentiated Caco-2 cells as a model for enterocytes in comparison to enterochromaffin QGP-1 cells after stimulation with cinnamaldehyde and 18 naturally occurring structurally related compounds by means of a serotonin ELISA. Stimulation with CA induced a dose-dependent increase in serotonin release starting from 0.5 mM, with a larger effect in Caco-2 compared to QGP-1 cells. In addition, further 18 compounds were tested at a concentration of 0.5 mM to investigate the impact of different structural characteristics of CA on serotonin release in Caco-2 cells. As a result, strongest serotonin release in Caco-2 cells was determined after stimulation with compounds known to activate TRPA1: coniferylaldehyde induced the strongest response with a 28.0±3.31 fold increase, followed by the parent compound CA (12.0±2.17), and cinnamyl alcohol (6.68±1.08), alpha-methyl-cinnamaldehyde (6.59±0.93), and eugenol (5.09±0.91), pointing to an involvement of the compounds ability to activate TRPA1. TRPA1 gene expression in

Caco-2 cells was confirmed by means of qRT-PCR. In addition, blocking of TRPA1 using 30 μM AP-18 significantly reduced the CA induced serotonin release by 30.0±5.24 %, confirming a TRPA1-dependent component in serotonin release by Caco-2 cells.

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P45

The X-ray structure of gurmarin provide new insights into amino acid residues essential for inhibition of the rat sweet taste receptor

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Gurmarin is a polypeptide isolated from the Indian plant Gymnema sylvestre, which specifically suppresses sweet taste in rodents without affecting responses to other basic taste stimuli, such as HCl, NaCl, and quinine. Although the exact mechanism of gurmarin inhibition is not known, it has been shown that gurmarin acts via the T1R2/T1R3 sweet taste receptor. The gurmarin molecule is made of 35 amino-acid residues and three intramolecular disulfide bridges. We report herein the 1.45 Å X-ray structure of gurmarin heterologously produced using the yeast Pichia pastoris. The structure revealed a typical knottin fold, which is compared with previously reported NMR solution structures. The atomic structure at this resolution allowed us to highlight a flexible region involving hydrophobic amino acid residues previously identified as a putative binding motif for the rat sweet taste receptor. By combining cellular based receptor assay and site-directed mutagenesis of gurmarin, we revealed that several amino acid residues located in this hydrophobic cluster of gurmarin severely affect rat sweet taste receptor inhibition. This study demonstrates that gurmarin can be used as a worthwhile tool to decipher the mechanism of sweet taste inhibition.

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P46

Expression and characterization of the human sweet taste receptor expressed in a mammalian inducible cell line

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Sweet taste perception is mediated by a heterodimeric receptor composed of the two distinct protein subunits, TAS1R2 and TAS1R3. TAS1R2 and TAS1R3 subunits are members of the small family of class C GPCRs. Class C GPCRs share a large N-terminal domain (NTD) linked to the heptahelical transmembrane domain by a cysteine-rich region. TAS1R2/TAS1R3 is the primary receptor for a diverse range of sweet compounds including natural sugars, sweet amino acids, artificial sweeteners and plant sweet-tasting proteins. In order to understand the molecular mechanisms that govern receptor – ligand interactions and the relative contribution of the two subunits to the detection of sweet compounds, we overexpressed TAS1R2/TAS1R3 using a stable tetracycline-inducible HEK293S cell line. Each TAS1R2 and TAS1R3 subunit were engineered by inserting two different N-terminal tags to allow the purification and detection of heterodimeric TAS1R2/TAS1R3 receptor. The functional activity of TAS1R2/TAS1R3 in heterologous HEK293S cells was analysed using calcium assay. A three-step affinity purification method was employed to purify the solubilized TAS1R2/TAS1R3 heterodimer. SDS-PAGE analysis showed that the receptor was pure. Circular dichroism demonstrated that the purified heterodimeric receptor was properly refolded and has expected secondary structures. Ligand binding was quantified using an intrinsic tryptophan fluorescence assay and revealed that solubilized TAS1R2/TAS2R3 was able to bind sucralose with an affinity in the high micromolar range. These results pave the way for future biophysical studies including NMR spectroscopy and electron microscopy.

P47

Role of chemokines in the murine olfactory system

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Neurogenesis in the central nervous system is increased in particular by injuries, e. g. as a result of a stroke. Nevertheless, the regeneration is usually insufficient what entails in many cases to long-term interferences. A better understanding of the underlying physiological processes is indispensable for the specific improvement of the treatment. The olfactory

system can be completely regenerated within 4–8 weeks after destruction of the olfactory neurons and is ideally suited to study proliferation and differentiation of neurons.

In the central nervous system the chemokine-receptors CXCR4, CXCR7 and their ligand CXCL12 (SDF-1) are localized especially in neurons, astrocytes and microglial cells, and are up-regulated in response to injuries. Levels of CXCR4 and CXCL12 in the olfactory epithelium seem to be higher than in the brain, but decrease throughout aging. CXCR4 is preferentially expressed in proliferative globose-basal cells, while CXCR7 is expressed in non-neuronal sustentacular cells. This is in contrast to the situation in most other tissues, where both receptors seem to be co-expressed in the same cell types. Interestingly, knock-out of CXCR7 in sustentacular cells has nevertheless marked influence on the number of mature neurons and immature CXCR4 expressing precursor cells. We currently analyze the role of the chemokine receptors and the ligand CXCL12 in specific genetically modified animal models.

In order to induce neurogenesis, we injected adult mice at the ages of 2, 6 and 18 months with Methimazole to destroy the intact epithelium, and analyzed the tissue after injection. CXCR4 in proliferative progenitor cells is highly upregulated 14 to 28 days after damage of the epithelium, suggesting a link between the CXCR4-CXCL12 axis and the regenerative and proliferative capacity of the olfactory epithelium.

P48

Analysis of the role of Ncam1a in the development of the taste sensory organ in zebrafish.

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We have recently shown using in vivo imaging in zebrafish that newly forming taste bud cells slither to join placodally forming organs (Soulika et al, 2016; Kapsimali, 2017). We have extended our observations to analyse the motility behaviour of cells within the taste bud organ. We have found that taste bud cells show short-scale rotations (4 animals, 83 cells).

We have made the hypothesis that cell adhesion is a key process in the assembly of sensory cells into a functional organ. Among the cell adhesion molecules differentially expressed in the zebrafish taste bud cells is Ncam1a. We have generated a CRISPR/Cas9 ncam1a null stable line and perform in vivo imaging in these mutants. Our results on the role of ncam1a in taste bud cell adhesion will be discussed in the ECRO2018 meeting.

Soulika et al, 2016 Development, doi: 10.1242/dev.134817.
Kapsimali, 2017 Development, doi: 10.1242/dev.148122.

MK is INSERM CR researcher.

P49

Calcium signals in taste-bud like 3D cultures.

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Taste buds are composed of 50–100 cells located in specialized papillae in the tongue. Of the four different cell types present, type II "receptor" cells are responsible for sweet, bitter, and umami taste transduction. They get in contact with flavours through an apical pore; primary signal transduction leads to intercellular communication via ATP and neurotransmitter release, which in turn activates gustatory nerves. Bitter substances are detected upon binding to one of 25 TAS2R-type receptors and production of intracellular Ca2+ signals via different pathways. Ca2+ changes were previously measured in lingual preparations or in isolated taste bud cells. However, both approaches are not suitable for high throughput analysis of taste compounds activity and they are often based on rodent tissues. Our purpose was to develop new human 3D-cell culture systems to test the response of taste cells to flavours in a more physiological context. We here report the successful generation of two different 3D cell cultures of immortalized cell lines derived from human fungiform papillae (Hocheimer et al., 2014) and the visualization of spontaneous and stimulus-dependent Ca2+ transients from these cultures in perfusion. To this end, we employed a stably proliferating cell line (HTC-8), which was transduced with the Ca2+ sensor G-GECO. Since HTC-8 cells express 13 different TAS2R types, they react to bitter compounds by Ca2+ signals. HTC-8-GECO cells were cultured in 3D as spheroids or in special polymer-based microcavities. In both systems, live Ca2+ imaging performed with confocal and light-sheet microscopy, revealed two major activity patterns: (i) mostly weak spontaneous Ca2+ transients upon perfusion with control solution and (ii) strong specific Ca2+ transients upon the addition of the bitter compound salicine in a subset of cells. The majority of cells responding to the compound were those showing a high activity already in control.

P50

Investigation of Ca^{2+}–mediated signaling in mouse vomeronasal sensory neurons

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In mammals, the vomeronasal system detects a large number of semiochemicals. These cues convey information about sexual, social and reproductive status and thus regulate behavior. Within the vomeronasal organ, vomeronasal sensory neurons (VSNs) translate chemosensory information into electrical activity which is processed in the brain. This signal transduction starts with activation of G-protein coupled receptors in VSN microvilli. A complex biochemical cascade is triggered leading to generation of different messenger molecules through phospholipid turnover. Ultimately, diacylglycerol-dependent gating of a Ca^{2+}–permeable ion channel is thought to complete signal transformation. However, our understanding of many basic VSN signaling mechanisms is still rather fragmentary. Using photoactivatable chemical constructs, we investigate the effects of Ca^{2+} influx into different regions of the cell. Focal laser-assisted uncaging experiments with o-nitrophenyl-EGTA allow subcellular control of Ca^{2+} concentrations at high spatiotemporal resolution. Experimental focus is placed on Ca^{2+} signal profiling within various neuronal compartments. Combining Ca^{2+} uncaging, Ca^{2+} imaging, and whole-cell patch clamp recordings, we generate a detailed activity map of Ca^{2+}-mediated signaling in mouse VSNs.

P51

How inflammation interferes with vomeronasal chemoreception in pigs: an immunohistochemical study

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The inflammation of the vomeronasal organ (VNO) has been recently associated with intraspecific aggression in domestic animals, as the consequence of an impairment of pheromones' detection in the affected subjects. The aim of this study was to explore the molecular and cellular changes that happen in the VNO during vomeronasalitis, to understand the mechanisms with which this pathology influences animal behaviour. Seventy-six VNOs were sampled from 38 six-month-old pigs and submitted to haematoxylin and eosin stain, anti-Gαi2 protein and anti-odorant binding protein (OBP) immunohistochemistry. Vomeronasal sensory epithelium (VNSE) inflammation was classified according to its intensity in absent, weak, moderate and strong. The number of VNSE Gαi2+ cells was counted in $1mm^2$ and VNSE thickness was measured on VNO microphotographs. As the OBP was located in VNO glands, its expression was counted as the percentage of positive pixels on the total. Statistical analysis was performed to compare Gαi2 and OBP expression and VNSE thickness to inflammation intensity. Of the 76 VNOs, 13 (17%) were healthy, 31 (41%) presented a weak chronic inflammation and 32 (42%) a moderate inflammation. VNSE Gαi2 expression and VNSE thickness were significantly reduced during inflammation ($p<0.05$) and accordingly to its intensity ($p<0.05$). OBP was more expressed in inflamed VNO compared to healthy ($p<0.05$), with no difference regarding vomeronasalitis intensity. These results suggest that porcine vomeronasalitis affects VNO function reducing Gαi2 protein expression and inducing vomeronasal neurons' death, altering in this way pheromones' detection. Inflammation effects on OBP should be still investigated, as its role in inflammation is not clear. To conclude, our study shows for the first time that an inflamed VNO has fewer receptors than a healthy one, being thus less able to play its key-role in animal chemical communication and behaviour.

There is no external funding source to declare.

P52

Secretoglobins as a novel class of Pheromone-Binding Proteins: The case of cat Fel d 1

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Pheromone-binding proteins of mammals are lipocalins, such as OBPs and MUPs. This study addresses the question whether secretoglobins, structurally different from lipocalins, may also have a role in chemical communication and pheromones' binding. Our model is secretoglobin Fel d 1 previously associated with cat sex and behaviour. First, bioinformatic analyses revealed structural similarities between Fel d 1 and the mouse Androgen Binding Protein (ABP), known for its role in intraspecific communication via steroid binding and mate selection. We observed strong phylogenetic relationships between the two proteins, functional residue conservation and good structural superimposition highlighting similar binding clefts tuned to some sexual steroids; in-silico docking simulations have further suggested interactions of Fel d 1 with some pheromones. In-vitro spectrofluorimetric ligand-binding assays using the probe 1-N-phenylnaphthylamine, recombinant Fel d 1, 18 fatty acids, and 12 steroids supported the in-silico predictions. We found strong interactions of oleic, linoleic and lauric acids, as well as androstenone with Fel d 1. Inhibition constants (Ki) were determined: particularly, lauric acid

(Ki=2.3 μM) and androstenone (Ki=2.2 μM) displayed higher binding affinities with Fel d 1. Finally, the presence of Fel d 1 in the glands of the vomeronasal organ (VNO) was assessed by immunohistochemistry analysis in 19 out of 25 cats. RT-PCR further confirmed these results by successful amplifications of transcripts of Fel d 1 genes in VNO tissue. RT-PCR products were sequenced for Fel d 1 identity confirmation, showing the existence of known isoforms in VNO. On the basis of the good affinity of Fel d 1 to pheromones and its expression in the VNO, we can reasonably propose that secretoglobins might be considered as a second class of pheromone-binding proteins in mammals.

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P53

Rhythmically discharging olfactory receptor neurons can encode the spatiotemporal characteristics of odor signals within complex fluid environments

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Sensory signals, including olfactory signals, are typically encoded by tonic receptor neurons. A significant portion of the olfactory receptor neurons in some species is intrinsically rhythmically active or 'bursting' (bORNS). Rather than phaso-tonically discharging to the odor onset as characteristic of tonic olfactory receptor neurons, in bORNs the frequency of their inherent burst is entrained by the intermittency inherent in turbulent odor plumes. Each bORN responds to a relatively narrow range of stimulus frequencies (intermittency) based on their inherent rate of bursting discharge and the phase dependency of their response to odor stimulation. Using computational and analytical approaches we have shown that heterogeneous populations of such uncoupled oscillatory neurons have the capacity to reliably encode the temporal properties of intermittent odor signals as long as seconds to many tens of seconds that characterize natural odor plumes. In the present study, we expand our current understanding bORN-based encoding by characterizing the molecular receptive range of bORNs, mapping their central projection, and beginning to identify the strategy for the synaptic processing of bORN-derived information at the first olfactory relay.

P54

Decoding odour source distance from turbulent gas plumes

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Odour-based navigation is essential for essential tasks such as locating food and mating partners and avoiding predators. However, turbulent airflow complicates source distance estimation in almost any habitat. Turbulence rapidly destroys any concentration gradient that might assist in source localisation. Moreover, the filamentous nature of turbulent gas plumes renders odour encounters highly intermittent. Odour-based navigation must therefore rely on features extracted from dynamical changes in odour concentration.

We designed a bio-inspired method to extract dynamic features of from recordings of turbulent odour plumes that correlate with source distance. Our analysis was based on freely available data that had been recorded using electronic gas sensors, placed in a wind tunnel at various distances from a gas source [1]. We employed signal processing that mimics the behaviour of an adaptive neuronal network, in that emphasises changes in the signal and deemphasises periods of little change. The analysis revealed that intermittent periods of continuously rising gas concentration, termed "bouts", are more frequent close to the source than they are at distant sample points. We found that bout-count statistics allow a reliable prediction of source distance. They outperform conventional spectral analysis of concentration time series by a large margin. Notably, bout-counts are, in essence, independent of gas concentration, and thus deliver distance estimations even when the concentration at the source is unknown.

Our results suggest a straightforward design of a neuronal circuit for source distance estimation. This circuit is compatible with circuitry described in the olfactory systems of vertebrates and insects, thus testable in computational models, and amenable to experimental validation.

[1] Vergara A, Fonollosa J, Mahiques J, Trincavelli M, Rulkov N & Huerta R (2013). Sens Act B Chem 185:462–477. http://doi.org/10.1016/j.snb.2013.05.027

P55

Copper ions potentiate the odorant responsiveness of narrowly tuned thiol-specific OR2M3 but not of broadly tuned thiol-selective OR2W1

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The recognition of key food odorants (KFOs) appears to be the most eminent capability of odorant receptors (ORs). However, the molecular mechanisms underlying a most sensitive detection of sulfur-containing KFOs are still largely unknown. Among the ~ 230 KFOs, which appear in foods above their odor threshold, sulfur-containing compounds engage an outstanding position, because of their extremely low odor thresholds. The observation, that copper ions can potentiate the responsiveness of odorant receptors (ORs) to thiols, led to the hypothesis that these receptors are metalloproteins. However, until now, only one human OR, OR2T11, has been reported to be activated by 2-methyl-2-propanethiol in a copper-dependent way. Combining homology modeling, docking studies, site-directed mutagenesis, and functional expression of recombinant ORs in a cell-based, real-time luminescence assay, we show that the narrowly-tuned receptor OR2M3 was activated by 3-mercapto-2-methylpentan-1-ol in a copper-dependent way, and that this activation depended on a "CSSH" copper interaction motive, which is conserved in subfamilies M, T, and V of family 2 ORs. We further show that the activation of broadly-tuned OR2W1, which does not carry the motive, by sulfur-containing KFOs did not depend on the presence of copper. In summary, our results suggest that a conserved copper interaction site decides on the ability to coordinate copper, and, by size restrictions of a binding pocket, may cause a thiol-responsive receptor to be narrowly or broadly tuned.

P56

Calcium-activated chloride currents in vomeronasal sensory neurons and their role in firing pattern.

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Vomeronasal organ (VNO) is a chemosensory organ and its main function is to detect pheromones that regulate the physiology and behavior of the animal. Vomeronasal sensory neurons (VSNs) mediate the signaling transduction in VNO using a PLC cascade that leads to increase of intracellular Ca^{2+}. VSNs express two calcium-activated chloride channels, TMEM16A and TMEM16B, but their physiological role is still unknown. It has been reported that single deletion of TMEM16B protein[1] or TMEM16A protein[2] from VSNs eliminates calcium-activated chloride currents without affecting the expression of other components of the transduction machinery. To clarify the role of calcium-activated chloride currents in VSNs, we used a general knockout mouse for the TMEM16B protein[3] and a conditional knockout mouse for TMEM16A (TMEM16A cKO). Surprisingly, and contrary to previous results[1], we found that calcium-activated chloride currents were still present in VSNs from TMEM16B KO mice; whereas they were absent in the TMEM16A cKO confirming our previous results. Then, we studied the spontaneous and evoked activity in VSNs from TMEM16A cKO using the loose-patch technique. We found that the mean frequency of spontaneous activity was not different whereas the firing pattern was altered in TMEM16A cKO, showing an inter-spike interval distribution with less burst activity compared with WT. Finally, we recorded responses to dilute urine and found an increase in spike frequency in both groups, indicating that neurons lacking TMEM16A are still able to activate the signal transduction cascade. Importantly, we observed significant changes in the pattern of firing activity, with VSNs from TMEM16A cKO firing with shorter intervals than WT neurons. These results indicate that TMEM16A modulates the firing pattern both during spontaneous activity and the response to physiological stimuli in VSNs.

1. Billig et al., Nat. neurosci., 14, 2011
2. Amjad et al., JGP, 145, 2015
3. Zhang et al., Neuron, 95, 2017

P57

Sensory characteristics of an Anti-HIV-1 drugs by human taste cell assays

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Human immunodeficiency virus-1 (HIV-1) disease is an infectious disease which affects people of all ages. In 2016, more than 36 million people (more than 2 million of them is under age 15) globally were living with HIV and almost 2 million people became newly infected with HIV-1. Ritonavir and Tenofovir Alafenamide (TAF) are the first-line treatments for HIV-1 infection, but non-compliance is high because of the strong unpleasant taste of these drugs. The taste of HIV drugs has been a longstanding concern in the treatment of HIV-infected patients both adults and children. One method for increasing compliance is to pharmacologically block noxious taste signals at the taste receptor level before these signals reach the brain. However, there is no information about which particular taste receptor(s) and other taste-related receptors are responsible for the aversive noxious taste of these drugs. In this study, we examined Ritonavir and TAF in cultured human fungiform taste (HBO) cells to identify the cellular and molecular targets in the mouth responsible for the unpleasant taste of these drugs. We demonstrated that Ritonavir and TAF activate bitter signaling pathways as well as TRPV1 and TRPA1 channels. Identifying the receptors

and signaling molecules that underlie the aversive tastes of oral pharmaceuticals being used to treat HIV diseases is a much needed first step to ameliorate their aversive tastes and promote compliance.

P58

Odorant receptor activation patterns of chloroanisoles, widespread off-flavours in wine and water.

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Wines are prone to contamination by a large variety of potent off-flavour-inducing compounds for example chloroanisoles, which may influence consumer behaviour. Until now little is known about their odorant receptor (OR) targets. 2,4,6-Trichloroanisole, however, has been described as a potent suppressor of olfactory signaling. Chloroanisoles, which represent typically musty or corky odour qualities, are among the most frequently found off-flavours in wine and water. Here we screened 600 OR variants with 2,4-Dichloroanisole, 2,6-Dichloroanisole, Trichloroanisoles, Tetrachloroanisole and Pentachloroanisole in a cell-based GloSensor â cAMP-luminescence assay, and identified a receptor that responded to a variety of anisoles. We also investigated the suppressive qualities of the chloroanisoles on olfactory signaling cascade with the assay GloSensor â cAMP, and a Ca^{2+}-imaging approach using HeLa/Olf cells expressing the CNGA2 channel. We discuss Chloroanisole-specific receptors as sensors for off-flavors in wine and water.

P59

Deorphanisation of the bitter taste receptors in the domestic dog (Canis familiaris).

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The TAS2R bitter taste receptor family plays an important role when animals make a decision on what to eat. Primarily thought to drive rejection, the activation profile of the T2Rs has been studied in several species but not in the domestic dog. Besides dog feeding, bitter tastes are also of interest as deterrents against unwanted ingestion of toxic ingredients by pets, as in the case of rodent poison and automotive antifreeze. In this study we identified and transfected all dog TAS2Rs into a heterologous cell-based assay system. We also selected orthologous human TAS2Rs for comparison where applicable. We screened a library of 36 structurally diverse bitter compounds and assessed their capacity to elicit calcium release in TAS2R transfected cells in comparison to mock transfects. Compounds were initially tested at three concentrations based on the maximum soluble concentration. Any receptor-compound combinations showing potential activity were retested in a full dose-response experiment where the compound was tested at eight concentrations. In many cases dog T2Rs were activated by typical bitter compounds used in human research. However, some differences were observed in the potency and efficacy of compounds when tested against both human and dog orthologs of a particular T2R. This work may help to identify potentially aversive compounds in dog foods with lower palatability, and also to identify compounds that perform well as deterrents to ingestion.

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P60

Generation of a taste organoid from non-human primate

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Taste organoid is a 3-D structured spheroid that consist of both stem cells and differentiated cells. Recently, we have generated taste organoids from mouse that has potential to fully differentiate into taste cells while some portion remain as stem/progenitor state. We found that taste organoids mimic taste cell proliferation and differentiation within the oral cavity so that could be a great in vitro model to study taste cells.

In this meeting, we will present our latest result of generating a taste organoid from Macaca fuscata (Japanese macaque). The monkey taste organoid presents spherical structure that is distinct from those of intestinal organoids. Transcriptome analysis has revealed that the organoid contains definitive taste markers such as sweet, umami, bitter taste receptor cells as well as signal transduction molecules that are known to be critical for taste signal transduction. Immunihitochemistry has been performed to show that the cells which are immunopositive for PLCbeta2 antibodies morphologically resemble to type 2 taste cells. Furthermore, we could obtain responses of tastant, such as sweetners or bitter compounds, when these tastants were applied to the organoid. We are currently examining whether this taste organoid could respond to artificial sweetners which rodents could not sense. Unlike the taste

organoids from rodents, this taste organoid could be maintained for more than 10 months and still have ability to show terminal differentiation into taste cells.

Therefore, we concluded that we have generated a new taste organoid from non-human primate.

P61

Odour sensing using arrays of odorant binding proteins

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Odorant Binding Proteins (OBPs) were investigated as sensing layers of chemical sensors, for the detection of organic compounds in vapour phases. OBPs are small soluble proteins present in high concentrations in the olfactory system of vertebrates and insects. They are attractive in the biosensor field since they can bind odorants and pheromones reversibly. They are resistant to high temperatures and protease activity and they can be easily expressed in large amounts. A number of recombinant OBPs from mammalian and insect sources were expressed, purified and characterised by ligand binding studies in solution. To create a sensor capable of detecting odorants in the vapour phase a transducer platform based on surface acoustic wave devices was chosen. The surface was modified by deposition of nanostructured diamond which was chemically modified so that a covalent linkage could be made with OBPs. The resulting structures were highly stable in air and responded reversibly to presentation of suitable odorants. The sensors had a lifetime between three months to one year dependent on usage. An array of eight OBPs were combined to form an array of sensors. The signals from the array were processed using principal components analysis and pattern recognition was implemented using a radial basis function neural network. The resulting system was able to discriminate and recognise in real time over fifteen different odorants ranging from single pure chemicals to complex mixtures such as tobacco or cigarettes.

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P62

Bitter compounds regulate mRNA expression of mucins and β-defensins in primary gastric cultures of lean and obese subjects

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[Objectives] Bitter taste receptors (TAS2Rs) are expressed on Paneth and goblet cells in the gut but their role on defensin and mucin expression has not been studied. Since obesity affects gut barrier functions, we investigated whether bitter agonists change innate immune responses in the human stomach of lean and obese subjects.

[Methods] Primary cell cultures of human fundus of lean organ donors (n=8) and obese patients (n=13) who underwent sleeve gastrectomy were treated with sodium benzoate (SB, 1 mM), and denatonium benzoate (DB, 0.1 mM – 1 mM) for 4h. mRNA expression of mucins (MUC1, MUC6, MUC13) and β-defensin 1 (DEFB1) and 2 (DEFB4A) was measured by RT-qPCR.

[Results] Basal mRNA expression of MUC6 was increased ($P<0.001$) in cultures from obese patients but no difference between both populations was observed for the other mucins and β-defensins. Treatment with DB, an agonist for several TAS2Rs (-4, 8, 10, 13, 39, 43, 46, 47) did not affect MUC1 and MUC6 expression but decreased MUC13 expression in both populations with a different sensitivity. In lean subjects, DB reduced MUC13 expression by 43% at 0.1 mM while in obese patients a comparable reduction was obtained with 0.5 mM DB. Furthermore, DB decreased DEFB1 ($P<0.05$) and DEFB4A ($P<0.01$) expression in obese but not in lean subjects. Similarly, the TAS2R14, -16 agonist, SB decreased DEFB4A expression in obese patients. To investigate whether TAS2R43 is involved in the DB-induced effects, results were compared between obese patients with (n=8) and without (n=5) TAS2R43. TAS2R43 did not mediate the effects on MUC13 but for the β-defensins the effect of DB was only significant in obese patients without TAS2R43, indicating that TAS2R43 is counteracting the effect of DB on other TAS2Rs.

[Conclusion] Bitter compounds regulate mRNA expression of mucins and β-defensins in human fundus. Obesity alters basal MUC6 expression and the sensitivity of bitter compounds on MUC13 and β-defensin expression.

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P63

Cadmium mediated olfactory dysfunction in fish

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The fish olfactory sensory receptor neuron (OSN) can respond to various aquatic chemical cues but may show adverse effects when exposed to various pollutants including heavy metals. The cytology based bioaccumulation of

heavy metal under transmission electron microscope [TEM: TECHNAI attached with X-ray microanalyzer (TEM-EDAX)] was emphasized to explore the ultrastructural consequences within ciliated OSN of Mastacembelus armatus (Lacepède, 1800) when exposed to heavy metal pollutants (e.g., Cd). Cadmium (Cd) is mostly accumulated within the cytoskeletal region (especially microtubules) of ciliated OSN. The dilated rough endoplasmic reticulum (rER), lysosomal diversity, fragmented microtubules as well as neurofilaments with vesicular crowding and docking, etc. are marked when Cadmium (Cd) level is 10.93% in compared to Below Detection Level (BDL) or minimal range of 0.06% - 0.09%. Apart from that, the synaptic region of ciliated OSN also shows vesicular distortions in response to large Cadmium (Cd) accumulation. The excess bioaccumulation of Cadmium (Cd) may cause membrane specific conformational changes in protein (?) and leads to interrupted olfactory synaptic transmission.

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P64

Receptomics to identify the targets for plant-based anti-obese compounds

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[Background] Obesity and consequently diabetes type II are among the biggest global health problems. Even though dietary and lifestyle changes may eventually reduce obesity for some individuals, new safe and more efficacious drugs are required for successful weight reduction and treatment of type 2 diabetes in a large proportion of obese individuals. It has been shown that various G-protein coupled receptors (GPCRs) expressed in various tissues are involved. GPCRs are important targets for food components. The members of this large family of membrane proteins are involved in virtually every physiological process.

[Aim and strategy] In this study, we will establish the influence of receptor/ligand interactions on the development of obesity and correlated T2DM. Next to this, we aim to identify food related compounds that can prevent or intervene in these chronic diseases. To accomplish this, we propose an integrated approach using state of the art technologies to test different food related compounds on a receptor level (receptor/ligand interaction), cellular level (in vitro experiments using relevant cell lines) and on organismal level (in vivo assays).

[Method] Identify food related compounds and receptors involved in obesity and related diseases, Screen food & plant extracts or compounds for receptor binding, energy metabolism and immune responses in various assays, Confirm results in in vivo assay with high-fat diet (HFD) fed mice, Status and future experiments.

A selection of 26 receptors based on literature and expression data was made. Initial receptor binding experiments using mushroom and plant extracts show specific receptor responses to serotonin, fatty acid, hormonal and cytokine related receptors. More compounds and extracts will be analyzed for receptor binding. Furthermore, compounds and extracts will also be tested in cell assays for metabolic activity and immune responses.

P65

Novel roles for the TRPM5 ion channel in regulating airway mucociliary clearance

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Mucociliary clearance (MC) is a major innate defense mechanism that removes pathogens from the airways. It depends on concerted ciliary beating and transepithelial ion transport. We showed previously that chemosensory tracheal epithelial cells, brush cells, sense "bitter-tasting" bacterial products and evoke protective respiratory reflexes utilizing the canonical taste transduction cascade (TRPM5, transient receptor potential channel 5). Here, we investigated the transcriptome of single tracheal ciliated and brush cells by single cell deep sequencing. The impact of various products from Pseudomonas aeruginosa (PA) on MC was estimated by cilia-driven particle transport speed (PTS) on explanted mouse tracheas. PTS was visualized by tracking the transport of dynabeads on the surface of the mucosa before and after application of six PA products: PQS, 2-AA, DHQ, HHQ, HQNO, and PyOC. Additionally, the influence of PQS and the bitter cascade agonist denatonium on transepithelial ion transport was examined with Ussing chamber experiments in murine isolated trachea. The effect on MC

differed between the investigated PA products. PQS, 2-AA and DHQ significantly increased the PTS. NGS of single tracheal epithelial cells revealed TRPM5 and PLC β 2 as hallmark genes for brush cells. The PQS-induced increase was abolished with the TRPM5 antagonist TPPO and reduced in TRPM5-deficient mice. HHQ and HQNO decreased PTS, while PyOC had no effect. Furthermore, PQS transiently increased net transepithelial ion current (Isc), whereas denatonium decreased Isc. This effect was dose-dependent and repeatable without affecting the amplitude of the response. TPPO significantly reduced the effect of denatonium, indicating involvement of TRPM5. Here, we show that bitter-tasting PA products alter both major MC components in a TRPM5 dependent manner. Thus, the TRPM5 ion channel is a novel key player in regulation of MC and might play an important role in protection against bacterial colonization.

P66

High-intensity sweeteners induce serotonin secretion via a T1R3-dependent pathway in human gastric cells in culture

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The role of sweet taste in energy intake and satiety regulation is still controversial. High intensity sweeteners (HIS) are thought to help reduce energy intake, although little is known about their impact on the satiating neurotransmitter serotonin (5-HT). In the gastrointestinal tract, 5-HT regulates gastric acid secretion and gastric motility, both involved in the regulation of food intake and satiety. Human gastric tumor cells (HGT-1) were treated with the HIS cyclamate, acesulfame potassium (Ace K), saccharin, sucralose, or neohesperidin dihydrochalcone (NHDC). For quantitation of 5-HT release into in the cell supernatant, the serotonin high sensitive ELISA (DLD Diagnostika, n = 3–6) was applied. ELISA techniques were also used to determine intracellular cAMP and ERK1/2 concentrations. mRNA expression of the sweet receptor subunit T1R3 was analyzed by qRT-PCR. Functional involvement of TAS1R3 was studied by using the T1R3 receptor antagonist lactisole, and a TAS1R3 siRNA knockdown approach. A stimulating impact on 5-HT release, compared to controls (100 %, $p<0.05$), was shown for cyclamate (50 mM, 157 ± 6.3 %), Ace K (50 mM, 197 ± 8.6 %), saccharin (50 mM, 147 ± 6.7 %), sucralose (50 mM, 194 ± 11 %), and NHDC (1 mM, 201 ± 13 %). Involvement of the sweet receptor subunit T1R3 in the NCS-evoked response was demonstrated by mRNA expression of TAS1R3, co-incubation experiments using the T1R3 receptor antagonist lactisole, and a TAS1R3 siRNA knockdown approach. Analysis of the downstream signaling revealed activation of the cAMP/ERK/Ca^{2+} cascade. Overall, the results obtained identify HIS as potent inducers of 5-HT release via T1R3 in human gastric parietal cells in culture and warrant in vivo studies to demonstrate their efficacy.

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P67

Niemann-Pick disease type C1 causes early decline in olfaction

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Niemann-Pick disease type C1 (NPC1) is a rare neurodegenerative disease often resulting in progressive degeneration and/or death of nerve cells. Its pathogenesis involves defective cholesterol biosynthesis and altered lipid homeostasis and storage.

Although several neurological symptoms (motor and sensory) are associated with the disease, how it affects olfaction is only beginning to be understood. Recent work on a NPC1 mouse model have shown that knocking out the NPC1 gene causes deficits in the olfactory system.

We addressed if olfactory deficits appear earlier in disease development compared to NPC1's more severe manifestations (loss of motor function) similarly to other neurodegenerative diseases (i.e. Parkinson, Alzheimer's). In which case, the sense of smell could be used as a diagnostic tool to monitor the onset of the disease. Thus, we investigated the olfactory system of the NPC1-knock out mouse model at different post-natal days to characterize the disease progression and to determine the time point at which the absence of NPC1 is altering olfaction both morphologically and functionally.

By combining immunohistochemistry and electrophysiology as well as behavioral tests, we found a progressive loss of mature olfactory receptor neurons in the olfactory epithelium of the NPC1-knock out mouse accompanied by a reduction in the magnitude of electro-olfactogram (EOG) odorant responses. In particular, we could observe a reduction in the number of olfactory marker protein positive neurons as early as post-natal day 36 (P36). This time point is also when the odorant-induced EOG responses showed a significant reduction. Also, to monitor motor function we performed the rotarod test and the NPC1-knock out mice

failed it only at a later time point while still showing a normal behavior at P36.

In summary, our preliminary results show that the olfactory deficits can be detected before more deleterious neurological symptoms at an early stage of the disease.

P68

Molecular basis of bitter taste receptor selectivity towards "bitter sugars"

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Human bitter taste receptors (hTAS2Rs) detect bitter compounds (Behrens and Meyerhof, 2006). These can have either toxic effects, such as the amygdalin of almonds (Bufe et al., 2002), or health benefits, such as the antipyretic effect of salicin (Akao et al., 2002). Intriguingly, the agonists of one member of the subfamily, hTAS2R16, consist of a sugar (generally glucose) attached to a hydrophobic moiety by a β-glycosidic bond (Behrens and Meyerhof, 2017).

Here we used homology modeling to generate the three dimensional structure of the receptor, followed by docking and multiscale molecular dynamics simulations (molecular mechanics/coarse grained approach (Leguebe et al., 2012)) to give insights into the specificity of hTAS2R16 for bitter β-glucopyranosides. We adapted our protocol from reference (Sandal et al., 2015).

Our simulations define the key interactions involved in ligand binding and provide an explanation for the heterogeneity of the hydrophobic moiety of the hTAS2R16 agonists.

P69

Mammalian taste cells express functional olfactory receptors

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The peripheral taste and olfactory systems in mammals are separate and independent sensory systems. In the current model of chemosensation, gustatory and olfactory receptors are genetically divergent families expressed in anatomically distinct locations that project to disparate downstream targets. Although information from the two sensory systems merges to form the perception of flavor, the first cross-talk is thought to occur centrally, in the insular cortex. Recent studies have found that although gustatory and olfactory receptors were named for their role in detecting tastes and smells, they are expressed throughout the body and serve as chemical sensors in multiple tissues. Olfactory receptor cDNA was detected in the tongue, yet no report has demonstrated the presence of physiologically functional olfactory receptors in taste cells. Here we show that olfactory receptors are functionally expressed in taste papillae. Both cultured human fungiform taste papilla cells and mouse taste papilla cells responded to odors and blocking the olfactory transduction component adenylyl cyclase eliminated these responses leading us to hypothesize that the gustatory system may receive olfactory information in the periphery. These results provide the first direct evidence of the presence of functional olfactory receptors in mammalian taste cells. Our results also demonstrate that the initial integration of gustatory and olfactory information may occur as early as the taste receptor cells.

P70

High-throughput optical tools for discovering molecular identity of physiologically-distinct neuronal populations

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Molecular markers have been a powerful tool for labeling and exploring a specific neuronal population. In many cases, however, a single marker corresponds to several, tens, or even hundreds of physiologically-distinguishable cell types. In such cases, there may be incentive to discover more specific markers. One promising approach would be to profile neurons physiologically and then discover genes expressed in a chosen subset of neurons. However, the tools for proceeding from neuronal function to gene expression are less well-developed.

In the present study, we report an approach called photoactivated, intersectional physiology sequencing (PIPSeq) to identify genes expressed in physiologically-distinct cell types. In this approach, we record neuronal activity by large-scale calcium imaging, label neurons of interest by photoactivation, and subsequently profile mRNA expression of labeled neurons. We applied PIPseq to the challenge of mapping receptor-ligand pairings among vomeronasal sensory neurons. PIPseq delivered dramatic enrichment of selected neuron types, generating high signal-to-noise sequencing data even of rare (<1%) cell types. Using this approach, we

identified the molecular fingerprints of the subsets of sensory populations representing overlapping but discriminable chemoreceptive fields.

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THEME III – VERTEBRATE TASTE AND OLFACTION: CENTRAL MECHANISMS

P71

ABL1 is a novel regulator in the laminated olfactory bulb structure formation during the early development

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Interneurons in the olfactory bulb(OB) are continuously generated and migrated from subventricular zone(SVZ) in the brain during the postnatal stage, while mitral and tufted cells in the OB are mainly formed during the embryonic stage. These neurons in the OB have their determined positions, and which makes lamination structures, similarly in the cortex or hippocampus. Abnormality of this structure leads to functional deficits, but how this process is induced and controlled has been little studied. We here report that the expression and activation of ABL1, a non-receptor type kinase in interneurons showed strong correlations to the early development of OB interneurons. Active ABL1 expressed in progenitors of interneurons massively entered into the OB until the first week after birth. Furthermore, when the ABL1 expression was inhibited by the injection of virus containing ABL1 shRNA and GFP during the first week of the birth, the distributed ratio of GFP+ cells were about 20% accumulated in deep granule cell layer(dGCL) compared to CTL group. It suggests that ABL1 may play a critical role in formation of proper OB organization during the early OB postnatal development, such as in migration and integration. In summary, the expression and activation of ABL1 at the specific stage appeared crucial for proper neuronal migration and laminated OB structure formation during the early OB development.

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P72

Bursting and non-bursting mitral cells differentially entrain olfactory bulb-entorhinal circuits in neonatal mice

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During early postnatal development, most sensory systems are still immature. Rodents are blind, deaf and have limited sensorimotor abilities until the second postnatal week. As a notable exception the olfactory system is already fully developed, controlling mother-offspring interactions and neonatal survival. In contrast to other sensory systems, information from the olfactory bulb (OB) bypasses the thalamus and directly targets the lateral entorhinal cortex (LEC) in adulthood. During neonatal development LEC seems to act as a gatekeeper of prefrontal-hippocampal circuits, driving discontinuous oscillatory activity in both areas. It is, however, still unknown how the mitral-tufted cells (MTCs), the output neurons in OB, shape the functional communication within OB - LEC networks during postnatal development. To fill this knowledge gap, we combine patch-clamp recordings from identified MTCs with multi-site extracellular recordings from OB and LEC in neonatal mice in vivo.

Similar to in vitro conditions, neonatal MTCs show two types of firing in vivo: bursting discharges in theta frequency range alternating with periods of silence and non-bursting, rather continuous discharges. Temporal correlations between intracellular recordings and extracellularly recorded patterns of activity in the OB revealed that the firing of bursting MTCs is stronger timed by discontinuous theta band (4–12 Hz) oscillations when compared to non-bursting MTCs. Moreover, MTCs fire preferentially during theta bursts in LEC. These data give first insights into the role of MTCs and there axonal projections for the physiological signature of OB-LEC communication during early development.

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P73

Effect of early experience on behavioral and neuronal responses to con- and heterospecific odors in closely related Mus taxa

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Olfactory plasticity to social olfactory cues is limited to a critical period. The objective was to evaluate the influence of early olfactory experience on the behavioral and neuronal responses of males to con- and heterospecific odors of receptive females in two species, M. musculus (subspecies musculus, wagneri) and M. spicilegus, and thus to determine the potential role of epigenetic contribution in the formation of precopulatory isolation. Males were reciprocally cross-fostered shortly after the birth and were tested for response to con- and heterospecific urine odors of estrous females using two-choice tests at 70–85 days of age. Neuronal activity of non- and cross-fostered males was evaluated at 90–110 days of age in MOB and AOB to con- and heterospecific female odor using fMRI (MEMRI). Non-cross-fostered males of three taxa demonstrated a strong preference for odor of conspecific females compared to odor of heterospecific ones. Spicilegus-nursed musculus preferred odor of heterospecific females. Wagneri-nursed spicilegus did not demonstrate significant choice of con - or heterospecific female odor. The level of MRI signal obtained from the evaluation of manganese accumulation in AOB neurons was significantly higher when the odor of conspecific estrus females was exposed, compared to urine exposure of heterospecific females. The response pattern changed to the opposite in males raised by heterospecific females. The maternal environment, including odor, had a greater effect on the level of MRI signal in the AOB than the genetic relationships of the recipient and the donor of the odor stimulus. Behavioral and neuronal responses to con- and heterospecific odors changed in closely related Mus taxa as a result of early experience. We demonstrated importance of early learning in mate choice in adulthood in mice and the possibility of epigenetic contribution in the formation of precopulatory isolation. Supported by the Russian Science Foundation grant №16-14-10269.

P74

A novel multi-glomerular domain in the mouse olfactory bulb defined by the calcium channel Cav2.1

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This study investigates the expression of the P/Q-type voltage-gated calcium channel subunit Cav2.1 in the mouse MOB and MOE using immunohistochemistry, immunoelectron microscopy, and RNA scope in situ hybridization methodology. Our experiments provide several converging lines of evidence indicating that Cav2.1 represents a novel candidate for olfactory signal transmission in a previously unknown subset of MOB glomeruli. The main findings of this study are: (1) Cav2.1 expression in the MOB is limited to a unique subset of glomeruli mainly located in the dorsocaudal and medial aspects of each olfactory bulb. (2) Cav2.1 localizes to the presynaptic axon terminals of OSNs targeting these glomeruli. (3) Both Cav2.1 protein and its corresponding mRNA Cacna1a also localize to a defined subpopulation of OSNs in the dorsal and medial MOE, indicating a distinct MOE-to-MOB topography of Cav2.1+ OSNs. (4) Cav2.1 expression demarcates a previously unknown multiglomerular domain in the MOB, perhaps even a novel olfactory subsystem that is characterized by the expression of OMP, Cnga2, and Pde4a. (5) This system is distinct from several cGMP-dependent olfactory subsystems that express type-D and type-G receptor guanylate cyclases or Trpc2 and the soluble guanylate cyclase Gucy1b2. Our work provides new insight into the architecture, organization, and projection targets of distinct mouse olfactory bulb glomeruli.

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P75

Amygdala corticofugal input shapes mitral cell responses in the accessory olfactory bulb

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Interconnections between the olfactory bulb and the amygdala are a major pathway for triggering strong behavioral responses to a variety of odorants. However, while this broad mapping has been established, the patterns of amygdala feedback connectivity and the influence on olfactory circuitry remain unknown. Here, using a combination of neuronal tracing approaches, we dissect the connectivity of a cortical amygdala (PmCo) feedback circuit innervating the mouse accessory olfactory bulb (AOB). Optogenetic activation of PmCo feedback mainly results in feed-forward mitral cell (MC) inhibition through direct excitation of GABAergic granule cells (GC). In addition, LED-driven activity of corticofugal afferents increases the gain of MC responses to olfactory nerve stimulation. Thus, through corticofugal pathways, the PmCo likely regulates primary olfactory and social odor processing.

P76

BOLD fMRI response to taste and food-related odor stimuli in awake mice

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Previous studies have shown that sweetener solutions can condition odor preferences in rodents. However, it is unclear where brain regions are activated by conditioned odor after taste-odor learning. To examine this question, in this study, we established a blood oxygen level-dependent (BOLD) functional MRI (fMRI) method for the responses to odor stimulation and taste stimulation in awake mice. The following activated brain regions were observed. By orthonasal odor stimulation, olfactory tubercle, amygdala, somatosensory cortex and hippocampus were activated. By intraoral administration of odor solution, piriform cortex, olfactory tubercle, somatosensory cortex, amygdala, hippocampus and hypothalamus were activated. By intraoral sweet taste stimulation, insular cortex, amygdala and hippocampus were activated. These results indicate that we constructed an awake fMRI system to confirm the responses to gustatory and olfactory stimuli in mice. In the future, we would apply these methods to identify the brain regions that respond to the intraoral administration of conditioned preference odor in taste-odor learned mice.

P77

Trpc5 expression in dopamine neurons of the hypothalamic arcuate nucleus

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Transient receptor potential (TRP) ion channels have been detected in neurons that are part of the neural network controlling olfactory-encoded reproductive physiology and behavior. During early pregnancy, a decline in dopamine release from hypothalamic arcuate (ARC) neurons is required to elevate prolactin levels, thereby stimulating a hormonal pathway preparing the uterine endometrium for implantation of a fertilized ovum. A depolarizing TRP-like channel has been suggested to play a role in dopamine ARC neuronal firing. Here, we identify the canonical Trpc5 channel in dopamine ARC neurons by performing double-labeling immunohistochemistry using specific antibodies that recognize tyrosine hydroxylase (Th) to identify dopamine neurons and Trpc5. Our results revealed that the vast majority of Th+ ARC neurons exhibit Trpc5 protein expression. We further evaluated Trpc5 expression using three distinct Trpc5 mutant mouse strains: the novel Trpc5^{L3F1} mouse harboring the L3F1 mutation containing a floxed exon 4 followed by an expression cassette interrupting the intron sequence between exons 4 and 5; and the previously reported Trpc5-E4$^{-/-}$ and Trpc5-E5$^{-/-}$ mice, in which either exon 4 (E4) or exon 5 (E5) is deleted. We also assessed Trpc5 expression in these mutant strains using RNAscope fluorescence in situ hybridization and polymerase chain reaction (PCR) analyses of genomic DNA. Together, these experiments revealed that the insertion in the L3F1 allele results in a hypomorphic mutation that causes Trpc5 knock-down with strongly reduced RNA and protein expression. Interestingly, the Trpc5 mutant females display severe impairments in their reproductive capabilities implicating a crucial role of Trpc5 in dopamine ARC neuronal activity.

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P78

Grehlin concentrations correlate with hunger and craving ratings following oral ingestion of macronutrients

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[Objectives] We investigated the effects of oral ingestion of different nutrient solutions on olfactory, cognitive, metabolic and psychophysical function and the role of grehlin for the prediction of hunger and food craving.

[Experimental methods] Twenty healthy men participated in our study employing a double-blind, cross-over, repeated measurement design (four different study days). Each day participants received, one of three isocaloric (protein, carbohydrate or fat 600 kcal, 1,500mL) solutions or a placebo. Olfactory and cognitive tests were conducted three times per day. Psychophysical and metabolic function tests were performed 7 times on each examination day (observation period:−60min, 0 = solution intake, +60, +120, +180, +240, and +300min). Data were subjected to analyses of variance (ANOVA) with time and solution as within-subjects factors. Pearson correlation coefficients were calculated to estimate the correlation between ghrelin concentrations and their hunger and food craving.

[Results] Odorants were perceived as more unpleasant directly after ingestion of the macronutrient solutions. Ratings of hunger and food craving significantly differed over the observation period with lowest ratings following application of the protein solution. We observed a significant positive correlation of active grehlin with hunger and fat, protein and sweets craving for each nutrient solution. Active grehlin significantly correlated with carbohydrate craving for carbohydrate and fat solution and with vegetable craving for fat solution only.

[Conclusions] The significant correlations of active ghrelin concentrations with hunger and craving ratings recommend ghrelin as predictor of these physiological measures.

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P79

Functional and morphological diversity of projection neurons in the olfactory bulb of larval Xenopus laevis

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The olfactory system is among the most ancient sensory systems and the blueprint of its neuronal circuitry is highly conserved across phyla. Olfactory sensory neurons detect odorant molecules and subsequently generate electrical activity patterns. The encoded information is then conveyed via the sensory neuron axons to the olfactory bulb. A population of projection neurons receives excitatory synaptic input of the sensory neuron axons and acts as first relay station of olfactory information processing. In mammalian olfactory systems, projection neurons were historically subdivided into mitral and tufted cells according to their morphology or localization in the olfactory bulb's histological layers. More recent research revealed that those populations are not only morphologically distinct, but also vary in terms of odor coding, axonal projection fields and their role in the circuitry. In earlier diverging vertebrates like amphibians, the clear distinction between projection neurons based on morphology and localization is more challenging. This is mostly due to high variability in shape and number of primary dendrites and less obvious cellular layering in the olfactory bulb.

In this work, we provide a functional and morphological characterization of the projection neuron population in larval Xenopus laevis. We used a broad spectrum of methods ranging from neuronal tracings over immunohistochemistry to functional calcium imaging. We found differences in projection neuron dendritic configurations, axonal projection patterns, neuronal marker expression and odor tuning. The larval amphibian olfactory system seems particularly suited to investigate projection neuron specialization from an evolutionary and developmental perspective.

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P80

Dynamic shift in innervation and response patterns of the amphibian olfactory bulb during metamorphosis

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The developmental shift from a water-smelling larva to an air-smelling adult requires extensive modifications to the olfactory system in many amphibians. We undertook a stage-by-stage survey of the anatomical changes and odorant response characteristics in the olfactory bulb of Xenopus laevis during metamorphosis. For this, we applied different neuronal tracing techniques, immunohistochemistry, and functional calcium imaging. In the larva, the olfactory epithelium in the principal nasal cavity detects waterborne odors and projects its axons to the ventral part of the olfactory bulb. During metamorphosis the principal cavity is remodeled and transformed into the adult air nose which sends its axons to a newly build enlarged dorsal olfactory bulb. The sensory cells lining the newly developing middle cavity – the adult water nose – extend their axons into the

ventral bulb. We found that the ventral bulb remains responsive to waterborne odors all throughout metamorphosis, which suggests a dynamic shift in innervation from the principal to the middle cavity without functional interruption. Furthermore, we investigated and characterized the subventricular zone, the putative origin of many bulbar neurons, and the development of the cellular components of the dorsal bulb. The changes taking place in the olfactory system in amphibians during metamorphosis provide valuable insights into the de novo formation and reorganization of functional neuronal networks. Adjusting sensory networks to new environmental demands plays a crucial role in evolution.

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P81

Vasopressin increases while oxytocin decreases olfactory nerve-evoked somatic Ca^{2+} responses in main olfactory bulb superficial granule cells

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In rodents, vasopressin (VP) and oxytocin (OT) both enhance recognition of known social odor signatures already at the level of the olfactory bulb (OB). Local application of VP in-vitro decreases the amplitude of evoked synaptic EPSPs in all tufted cells (TC) and 70 % of the mitral cells (MC). Projections of OB VP cells innervate the whole external plexiform layer, pass the MCs and reach the internal plexiform layer. Superficial granule cells (sGC) reside within this projection area, extending their dendrites in the external plexiform layer, where they inhibit TCs and MCs. To investigate if the depressing effect of VP on MCs and TCs could be mediated via excitation of sGCs, we performed two-photon population Ca^{2+} imaging in OB slices of juvenile rats loaded with the AM dye Cal520. We recorded Ca^{2+} responses in MC and GCs via electrical stimulations of the olfactory nerve (400 µA, 100µs). MC and GC responses could be differentiated via the area of the excited cell soma (346 .2 ± 91.5 vs. 39.3 ± 12.9 µm^2, n=16/N=2, $p<0.05$) and the half-duration of the Ca^{2+} responses (5.0 ± 0.3 vs. 2.0 ± 0.3 s, $p<0.05$). Indeed, bath application of 1 µm VP increased Ca^{2+} responses in sGCs (n=111/N=6, $p<0.01$). In detail, VP increased the amplitude to 122.2 ± 42.5 and the decay to 140.2 ± 77.5 % from baseline. Intriguingly, application of 1 µM OT resulted in a reduction of the amplitude and the integral of the Ca^{2+} responses (n=27/N=5, $p<0.05$) to 69.7 ± 19.7 and 72.2 ± 24.8 % from baseline, respectively. It remains to be elucidated whether these opposing effects are due to the activation of different receptors or if there are indeed differential effects of VP and OT on the same receptors.

P82

Social neuromodulation in the olfactory bulb: Suppression of olfactory nerve inputs to principal and vasopressin cells by vasopressin and serotonin

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Olfactory cues are used to recognize individuals in many mammals. Local administration of VP into the olfactory bulb (OB) enhances social recognition and decreased the spontaneous firing rate of mitral cells in-vivo in rats. Therefore it is suggested that VP facilitates network processing of social olfactory cues in the OB. To investigate VP effects on synaptic inputs to mitral and projecting tufted cells (TC), we performed whole cell patch clamp recordings in in-vitro OB slices of juvenile rats following electrical olfactory nerve (ON) stimulation. Bath application of VP (2 µM) did not significantly decrease the amplitude of ON-evoked EPSPs in mitral cells (n=10). In contrast, VP (1 µM) significantly decreased the amplitude of ON-evoked EPSPs to 54 ± 19 % of baseline in TC (n=6, $p<0.05$). To better understand the intrinsic source of VP release, we also recorded from eGFP-labeled bulbar VP-expressing cells. ON stimulation induced large IPSPs in VP cells (n=11, 11 ± 3 mV). Bicuculline blocked these IPSPs, unmasking EPSPs in most cells. Although VP (1 µM) decreased the amplitude of the IPSPs to 65 ± 17 % of baseline (n=6, $p<0.05$), additional inputs would be necessary to sufficiently excite VP cells. Centrifugal serotonin (5-HT) projections are promising candidates for that, as OB innervation of 5-HT fibers is most intense in the glomerular layer and 5-HT increases excitation of short axon cells as well as external TC. Further, 5-HT deficiency in rodents was shown to reduce social interaction behavior, which is a prerequisite of gaining olfactory social cues. So far we demonstrated that application of 5-HT (20 µM) decreased the amplitude of ON-evoked IPSPs to 72 ± 32 % of baseline in VP cells (n=7, $p<0.05$).

P83

OR37 ligands exposure reduces activation of the paraventricular nucleus of hypothalamus following anxiogenic behavioural tests

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Members of the evolutionary conserved OR37 family of mouse olfactory receptors are known to have an unusual direct projection to the paraventricular nucleus (PVN) of the hypothalamus, via the main olfactory bulb (MOB). Previous research has shown that the mixture of three OR37 ligands: pentadecanal, hexadecanal, and heptadecanal, were effective in reducing activation of the neurons in the PVN following novel cage exposure. This effect suggests that the OR37 ligands may play a role in reducing the stress axis response by a potential social buffering effect.

The aim of this study is to investigate whether this effect generalises to other anxiogenic environments. The results showed significant decreases in cFos expression in the PVN for male and female mice exposed to the OR37 ligand mixture compared to the control, following open field and zero maze tests. OR37 ligand mixture exposure was also found to significantly increase the time spent in the centre of the open field arena, indicating an anxiolytic behavioural effect. Future work will focus on investigating the effects of OR37 ligands in other anxiogenic contexts and include other stress-related outputs, such as corticosterone level. A better understanding of the effects of OR37 ligands on the stress axis may have future applications in ameliorating stress responses during husbandry and laboratory procedures, with consequent animal welfare benefits.

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P84

The function of gonadotropin-releasing hormone (GnRH) neurons in olfactory computations

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Animals interact with and respond to their environment by processing sensory cues. However, depending on its internal state (e.g. satiety/hunger), the same sensory input can produce different behaviors. Neuromodulators are crucial in modulating the internal state of the animal. Gonadotropin-releasing hormone (GnRH) is a conserved neuropeptide and, modulates feeding and reproductive behaviors. It is produced by discrete neuronal populations located in the terminal nerve region of olfactory bulb (OB) and hypothalamus and is dispersed widely throughout the brain. Studies suggest that GnRH modulates the basal olfactory response by altering the synaptic properties of olfactory receptor neurons. However, the specific role of GnRH neuromodulation in olfactory information processing is not well understood.

To address this, first, we created a transgenic zebrafish line that labels the terminal nerve GnRH3 neurons with Gal4 transactivator. Genetic ablation of these neurons led to a significant decrease in feeding and food odor induced behaviors. Next, we expressed a genetically encoded calcium indicator (GCaMP6s) in GnRH3 neurons and imaged their activity by using two-photon microscopy. In line with previous studies, we observed that olfactory bulb GnRH3 neurons exhibit a high level of ongoing spontaneous activity. Moreover, we showed that GnRH3 neurons respond to a diverse set of olfactory stimuli and with strong preference for food related odors. The odor responses of GnRH3 neurons are sexually dimorphic and strongly modulated by physiological states (satiety/hunger) of the animals. By using a combination of optogenetic stimulation, imaging and electrophysiology, we are currently investigating how GnRH3 neurons are connected to the rest of the OB circuits and modulate (and are modulated by) the activity of the OB neurons. Our results suggest a central role for the GnRH neuromodulation in the regulation of OB activity, odor coding and olfactory behaviors.

P85

Temporal constraints of figure-background segregation of odors

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Several recent studies have analyzed the time required for rodents to make decisions based on olfactory cues and found that odors can be discriminated remarkably fast (within a single sniff). Albeit the general agreement on the speed at which olfactory discriminations can be made, studies have reached rather conflicting results about how the difficulty of discrimination affects its speed. These studies have all used behavioral paradigms in which mice are required to discriminate between a rather small set of simple isolated stimuli. Here we analyzed the temporal constraints of odor guided decisions in a more complex task – detecting target odorants against varying backgrounds. Mice were presented with odorant mixtures and were trained to report the presence/absence of target odorants in a 2 choice task. Mice reported correctly over 80% of the trials with decision accuracy decreasing when the number of mixture components increased. The mean reaction time was 470 ms – almost twice

the reported figures for simple odor discrimination. For both easy and difficult trials, mice benefitted from sampling the odorant mixture longer, but as the number of mixture components increased, mice had to sample the mixture longer to achieve similar relative performance. Each additional component required about 30 ms longer sampling. These findings indicate that olfactory figure-background segregation requires longer stimulus sampling than simple 2 odor discrimination, and that a tradeoff between speed and accuracy is clearly evident in this task.

P86

Early olfactory experience affects perception of predator odours in the house mouse

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Unique amino acid L-felinine and its volatile derivatives may be used by the house mouse to recognize potential predators, their physiological status and may affect reproductive output in mice (Voznessenskaya, 2014). Aim of our study was to examine whether early olfactory experience (EOE) of mice with cat chemosignals may affect sensitivity to target odours later in adulthood, modulate behavioural or neuroendocrine responses and whether these changes in sensitivity correlated with neural activation in olfactory bulbs. Olfactory thresholds to cat urine and L-felinine were measured with an automated olfactometer (Knosys, USA). Fecal glucocorticoid metabolites and plasma corticosterone were monitored using an ELISA technique. Behavioural patterns were analysed using an open field paradigm (2 different modifications). We performed immunohistochemical studies to identify neural substrate involved in reception and analysis of L-felinine and derivatives. Exposures of mice to cat chemosignals (urine/felinine) significantly lowered the olfactory thresholds ($n=10$, $p< 0.01$) which facilitates chemosensory detection of the predator. EOE with cat urine/felinine also decreased ($n=22$, $p< 0.01$) patterns of passive-avoidance behaviour to cat odours and significantly elevated investigatory activity ($n=22$, $p< 0.01$) pointing to plasticity of the behavioral responses to cat chemical signals in mice. At the same time corticosterone response to cat urine/Felinine stayed unchanged ($n=10$, $p< 0.01$) indicating the innate nature of the response. We recorded specific pattern of activation in accessory olfactory bulb (AOB) in response to stimulation with L-felinine(0.05%). EOE with L-felinine (0.05%) caused significant increase in number of Fos-positive cells in AOB ($n=8$, $p < 0.01$), in the size of activated area ($n=8$, $p<0.001$) as well as we recorded an increase in density of Fos-positive cells ($n=8$, $p < 0.05$) in response to stimulation with L-felinine.

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P87

AON top-down projections modulate olfactory bulb output activity

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The olfactory bulb (OB) is the target of massive cortical top-down projections from the anterior olfactory nucleus (AON) whose role in modulating early olfactory processing remains elusive.

Here, we examine how top-down projections from the AON modulate output neuron activity from the OB using imaging, electrophysiological and optogenetic approaches in vivo. Optogenetic activation of AON axon terminals in the OB led to a significant decrease in mitral/tufted (MT) cell spiking in the absence of inhalation-driven sensory input. The population time course showed a fast reduction of MT cell activity during light stimulation that was followed by a long lasting increase, reminiscent of OB offset responses. Since sensory input was shown to trigger AON feedback activity to the OB, we also tested for AON modulation effects during inhalation of clean air as well as during odor presentation. In both conditions, MT cells showed a decrease in activity similar to that observed in the absence of sensory input, arguing that our light stimulation protocol already strongly activated AON OB axons. Averaged normalized sniff-triggered spike histograms showed a decrease in both baseline and peak spike rate, consistent with an AON mediated effect on odor sensitivity rather than an influence on signal-to-noise ratio. Furthermore, AON stimulation effects were independent of the strength and polarity of the odorant response.

Preliminary imaging experiments yielded similar results: AON stimulation caused a decrease in spontaneous as well as odor-evoked MT cell Ca^{2+} transients and so far, no odor specific effect could be observed.

Our results suggest that AON activation is capable of reducing OB output activity independent of sensory input strength. Future experiments will focus on translating our findings in anesthetized animals to the awake condition.

THEME IV – HUMAN STUDIES

P88

The human olfactory cleft mucus proteome and its age-related changes

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Age-related decreases in olfactory sensitivity are often accompanied by a decrease in the quality of life. However, the molecular mechanisms underlying these changes are not well described. Inhaled substances including odorants are detected by sensory neurons in the olfactory cleft covered with a layer of mucus. This olfactory mucus is the first molecular machinery responsible for tissue protection and for detection of environmental odorants. Yet, little is known about the molecular identities of the actors because of the lack of information on the mucus proteome and its age-related changes. Here, we sampled human mucus from different nasal locations and from young and elderly subjects. The composition of the mucus was extensively analyzed by shotgun proteomic analysis for a vast array of proteins. We also explored correlations between the levels of each mucus proteins with the olfactory sensitivity of subjects. This analysis revealed previously unrecognized proteins with potentially important functions in olfaction. Taken together, this report describes the most comprehensive catalogue of the nasal mucus proteins to date, their positional and age-related differences, and candidate proteins associated with olfaction. This catalogue will provide fundamental information useful for future studies, such as identification of olfactory auxiliary proteins, causes of age-related declines in olfaction, and biomarkers for neurodegenerative disorders.

P89

Olfactory function and apathy as potential biomarker in patients with Parkinson's disease

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[Introduction] Parkinson's disease (PD) is a neurodegenerative disorder characterized by motor symptoms as bradykinesia, rigidity, tremor and postural instability. Additionally, PD is usually associated with non-motor symptoms (NMS) that include smell and taste dysfunctions, neuropsychiatric symptoms such as apathy, anxiety and cognitive impairment, sleep problems and autonomic dysregulation [1–2].

The aim of the study was first to investigate olfactory function, cognitive impairment, apathy and fatigue in PD patients in relation to healthy controls, and second to analyze the relationship between these NMS and the severity of motor symptoms in subjects with PD.

[Materials and methods] One hundred and forty-seven participants were enrolled (96 PD patients, mean age in years: 67.5, SD: 7.2; 51 healthy controls; mean age: 65.1, SD: 11.8). Olfactory function was evaluated using the Sniffin' Sticks test. The Montreal Cognitive Assessment (MoCA) was used to assess cognitive impairment. Apathy was examined by the Starkstein Apathy Scale (SAS) and fatigue was evaluated by the Parkinson's Disease Fatigue Scale (PFS).

[Results] PD patients showed severe impairment in olfactory function compared to healthy controls. Moreover, in PD patients apathy and fatigue scores were significantly increased, while MOCA scores were significantly decreased in comparison to controls. Multivariate linear regression analyses showed that both apathy and UPDRS were associated with olfactory function.

[Conclusion] Our results identified a greater level of apathy in PD patients affected by severe olfactory loss. Moreover, the present study confirms that alteration of olfactory parameters, such as odor threshold, identification, discrimination and TDI score, are related to other NMS.

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P90

Human chemosensory aggression signals augment aggressive behavior in response to frustration

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First results indicate that humans communicate aggression via chemosensory signals, however, it is unknown whether chemosensory aggression signals affect complex behavior. Thus, the current study investigates the effects of chemosensory aggression signals on aggressive behavior during frustrating social interactions.

A total of 84 individuals (42 female, 42 male) were confronted with fictional opponents stealing either 80% (frustrating condition) or 20% (control condition) of money the participants had worked for earlier. Participants could penalize their opponent by administering up to 10 drops of unpleasantly hot tabasco sauce (aggressive behavior). Upon deciding on the amount of sauce to administer, participants were exposed to aggression sweat, emotionless sweat, or clean air for 3 seconds (3-channel-olfactometer, flow rate: 46 ml/s), believing they were smelling their opponent's body

odor. Axillary sweat was sampled via cotton pads from 17 men responding aggressively to a frustrating computer game (aggression sweat) and playing a construction computer game (emotionless sweat). These donors featured an increase of anger (p = .02) and higher testosterone levels during frustration compared to the construction game (p = .08).

Following frustration, participants administered more hot sauce when exposed to aggression sweat compared to both emotionless sweat (p = .01) and clean air (p = .07). During the control condition participants also administered more hot sauce when exposed to aggression sweat compared to clean air (p = .03). Participants administered more hot sauce in the frustrating compared to the control condition ($p_s \leq .001$), irrespective of the chemosensory context.

Aggression sweat amplifies aggression following frustration, indicating an efficient chemosensory communication. As even in the absence of frustration chemosensory aggression signals foster aggression, they seem to override social information of other sources.

P91

Modulation of women emotional state by male axillary secretions depends on the phase of menstrual cycle

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Our earlier research showed that male axillary extracts (MAE) could shorten the length of menstrual cycle not only in women of reproductive age, but also in premenopausal women (Voznessenskaya, Laktionova, 2018). Several studies showed that MAE could modulate emotional state of women, but there are no investigations considering the influence of menstrual cycle phase of the test subjects. The aim of current study: to investigate the influence of MAE on the mood of women depending on the menstrual cycle phase. For evaluation of emotional state we used adapted for Russian population version (Osin, 2012) of Positive and Negative Affect Schedule (PANAS) (Watson, Clark, 1988) in comparison with visual analog scales (VAS). Axillary secretions were collected during 4 hours from 6 healthy heterosexual men (age of 31,8±4,7 years old) followed by extraction with ethanol; masking fragrance was added (Preti et al., 1986). Test subjects could not distinguish experimental and control samples by smell. Healthy female test subjects (n=36, age of 20,3±4,6 years old) completed VAS and PANAS before and one hour after beginning of exposure to MAE or control samples. Negative affect (NA) depended significantly on the phase of menstrual cycle of test subjects (p=0,035; n=32; F(1,28)=4,92, Mixed design ANOVA). Under MAE influence NA decreased in group of women in ovulatory & luteal phase (p=0,043, n=10, Wilcoxon test), but not in group of women in follicular phase (p=0,500, n=5) or control group (p=0,086, n=10). This result also confirmed for independent group of women in follicular phase of menstrual cycle (p=0,345, n=11, Wilcoxon test). VAS showed that women in ovulatory & luteal phase but not in follicular phase of menstrual cycle (p=0,893, n=5) were less «nervous» (p=0,017, n=10, Wilcoxon test) after MAE exposure relative to controls. Using two different tests we showed the significance of the phase of menstrual cycle for MAE mood modulatory effects.

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P92

Olfactory sensitivity evaluation of banana flavour by MS-GC-ODP technique in subjects tested by the "Sniffin' Sticks" extended test.

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Humans can accurately discern thousands of odorants, although there is a considerable inter-individual variability. Individuals can be classified as normosmic, hyposmic or anosmic, depending on their sensitivity or blindness. In this research we studied the olfactory sensitivity to the banana head-space as a complex odour mixture in a group of 40 subjects classified for their olfactory status, as normosmic, hyposmic or anosmic, by means of the "Sniffin' Sticks" extended test. The contribution of each single test (threshold, discrimination, identification) on the total TDI score was also evaluated. Using the coupled Mass Spectrometry-Gas Chromatography-Olfactometric Detection Port (MS-GC-ODP3) technique, the single components of the banana flavour mixture were separated, identified and verbally evaluated by each subject. All molecules detected by subjects were classified into one of the following three categories of flavours: fruity, floral and herbal.

The results show that the score of threshold, discrimination and identification contributed to TDI scores for 51.37%, 35.12% and 12.54%, respectively. TDI scores are linearly correlated with the number of fruity molecules detected from banana mixture. In particular, the isoamylacetate (IAA) molecule was smelled and properly identified in a higher number of subjects classified as normosmic, by total TDI, threshold and discrimination score, with respect to those classified as hyposmic. On the other hand, subjects who did not smell IAA (by MS-GC-ODP3), were able to correctly identify the banana flavour in Sniffin' Sticks tests.

In conclusion, our results show a direct relationship between the sensitivity to IAA, as well as that to fruity molecules of banana mixture, and the olfactory status, mostly determined by threshold and discrimination tests.

P93

Lavender ambient odour may affect human cortisol secretion in an age-dependent manner

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Lavender oil (LO) is traditionally used to relieve stress and anxiety. Meanwhile, LO may negatively affect working memory and attention in adults (Moss et al, 2003). In our earlier studies we observed positive effect of LO (~0.13 mg/m^3) on task performance in children in language test while in mathematics tests the effect of LO on task performance was negative (Rodionova, Minor, 2005). The aim of the current study - to evaluate the effect of LO (Sigma-Aldrich; ~0.13 mg/m^3) on salivary cortisol in children (10–11 years old) in comparison with college students (18–21 years old). Salivary cortisol is widely used as a biomarker of stress. In turn, stress is a major factor that affects memory retrieval processes. Saliva samples were taken every 15 min during control lesson (no odor, 45 min) and experimental lesson (LO or no odor, 45 min). All experiments were performed at the same time of the day. Salivary cortisol was monitored using an ELISA technique. We did not observe any significant sex differences of basal saliva cortisol in children though we observed considerable variation in basal saliva cortisol. We performed analysis depending on the basal level of cortisol. In children with low basal saliva cortisol (<20 ng/ml) exposure to LO caused a significant elevation of the hormone ($p<0.05$, Fisher test). In the group of children with normal or elevated salivary cortisol (>20 ng/ml) we observed a significant drop of the hormone ($n=8$, $p<0.05$, Wilcoxon test) in presence of LO. In college students we observed significant sex differences in basal saliva cortisol ($p<0.05$, Mann-Whitney test). However, LO did not affect salivary cortisol neither in women ($n=10$, $p=0.09$), nor in men ($n=10$, $p=0.88$, Wilcoxon test). Thus, we observed age related differences of LO effects on cortisol secretion. Taking these data into consideration, mechanisms underlying changes in task performance under LO influence could be different in children and adults. Funded RSF 16-1510312 to V.V.

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Peppermint ambient odour effects on cortisol secretion in humans depend on gender and age

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There is a growing set of evidences that ambient odors of essential oils have effects on human mood, behavior and physiological state. Earlier we showed that exposure to peppermint odor (PO) selectively improved performance of schoolchildren in standardschool tests which relied mostly on working memory (Rodionova, Minor, 2017; Voznessenskaya et al., 2018). A number ofstudies showed that PO may also improve working memory in adults. Theaim of the current study: to evaluate the effect of peppermint oil (Sigma-Aldrich; 0.13 mg/m3) on saliva cortisol in children (10–11 years old) in comparison with college students (18-21years old). Saliva cortisol was monitored using an ELISA technique. Individual saliva samples were taken every 15 minutes during control lesson (no odor, 45 min) and experimental lesson (PO, 45 min). All experiments were performed at the same time of the day. We used identical experimental design in schoolchildren and college students. Saliva cortisol was significantly lowered by PO in children of both sexes ($p<0.001$, $n=14$, Wilcoxon test) regardless of the initial basal level of saliva cortisol. In college students we observed sex differences in basal saliva cortisol: female students had significantly higher saliva cortisol level than male students ($p<0.05$, $n=10$, Wilcoxon test).PO caused a significant drop of saliva cortisol in female students ($p<0.01$, $n=10$, Wilcoxon test) but not in male students ($p>0.05$, $n=10$, Wilcoxon test).Sex differences in PO effects on saliva cortisol in adults require further investigations on anticipated differences at the behavioral level. Saliva cortisol is widely used as a biomarkerof stress. Stress is a very common event in educational settings which markedly impairs memory retrieval processes both in adults and in children (Vogel, Schwabe, 2016). Positive effect of PO on task performance in schoolchildren we explain by lowering of cortisol which facilitates memory retrieval

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Trial measurement of brain activity underlying olfactory–gustatory synchrony perception using event-related potentials from five female participants

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Temporal synchrony between odor and taste plays an important role in flavor perception. When we investigate

temporal synchrony between odor and taste, it is necessary to pay attention not only to physical simultaneity of the presentation of olfactory and gustatory stimuli, but also to the perceptual simultaneity between the two stimuli. In this study, we examined short - latency brain activity underlying synchrony perception for olfactory–gustatory combinations. While five female participants performed a simultaneity judgment (SJ) task using soy sauce odor and salt solution, single-channel event-related potentials (ERPs) were recorded at the position of Cz. In each trial, the participant was asked whether olfactory and gustatory stimuli were perceived simultaneously or successively. Based on the judgment responses acquired from participants (i.e., simultaneous or successive), ERP data were classified into two datasets. The means of ERPs from each participant were calculated for each type of judgment response, considering the onset of olfactory or gustatory stimuli (OERPs or GERPs, respectively) as the starting point. The latencies of the P1 component of GERPs were very similar between simultaneous and successive judgment responses, whereas the P1 amplitudes differed significantly. These results indicated that neural activity affecting SJ for an olfactory–gustatory combination is generated during a period of about 130 ms from the onset of gustatory stimulus. Thus, olfactory and gustatory information processing related to flavor perception (more specially, synchrony perception between odor and taste) might be initiated at a relatively early stage (ex. the thalamus) of the central pathway.

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Is there a chemical signal of friendship?

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Friendship is a fundamental characteristic of human beings. Friends influence the behavior, attitudes, personal live, thoughts and actions of each other and friendships are formed with similar individuals. A recent study found that the similarity of friends extent to their genotypes, which are more similar among friends than expected by chance. The question that is unanswered yet is, how individuals assess genetic similarity of another person. Here odour might play an important role as it is known that genetic similarity correlates with odour similarity. To find out whether odours might be involved in friendship formation or maintenance, we tested in a first step two hypothesis. First we analysed whether the chemical fingerprint of friends are more similar than average. Using gas-chromatography (GC) we characterized the chemical fingerprints of 174 students, which were previously asked whether friends of them were present in the same course. In case odours might be involved in friendship formation we expected to find friendship groups to be characterized by more similar chemical fingerprints. In a second step we conducted a two-step odour preference test with a subset of these students, in which we gave students first their own odour sample and the sample of an unfamiliar same sex individual and second we gave students the odour sample of their same sex friend and one of a same sex unfamiliar individual. Again if odours are involved in friendship formation or maintenance we expect individuals to significantly prefer the odour of their friend. Here we will present the results of the chemical analysis and the experiments and will discuss the implication of our findings.

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Smelling intentions. Can human chemosignals influence the perception of action intentions?

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Considering that agents are constantly embedded in social contexts and that social interactions require the ability to aptly make inferences on or judge the intentions of others through multiple sources of information, we set out to investigate whether and how odor stimuli could modulate the accuracy and the response times of the judgments discriminating the agent's motor intentions based on the presentation of temporally occluded videos, showing an agent (arm only) performing reach-to-grasp movements in individual (natural speed vs. fast speed) and social (cooperative vs. competitive) conditions. In Experiment 1, we assessed whether and how action intention judgements changed based on the exposure to a common odor or no odor. In Experiment 2, we compared how the common odor influenced the classification of cooperative and competitive action intentions with respect to a human chemosignal, masked with the same common odor. In Experiment 3, we contrasted whether and how human chemosignals collected in either cooperative or competitive situations could bias the detection of action intentions. Experiment 1 showed that the common odor had a facilitatory effect in detecting action intentions in blocks that presented a greater difficulty in the classification, suggesting a modulation of attentional resources. Surprisingly, in Experiment 2 and in Experiment 3 the cooperative human chemosignal enhanced the classification of competitive action intentions, suggesting that a response to a threating stimulus that might be due to the social distance between the recipient and the donor, i.e. being a stranger. These findings suggest that both common odors and human chemosignals influence the classification of action intention irrespective of the awareness of the olfactory signal. Such effects are interpreted in the context of the hierarchy of information conveyed via human chemosignals.

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Olfactory dysfunction under withdrawal syndrome in long-term heavy drinkers of alcohol. An impact of dual diagnosis.

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Long-term alcohol abuse results in the inhibition on neurons by ethanol. With withdrawal of alcohol exposure, low inhibitory activity ($GABA_A$) and increase of glutamate activity (NDMA) activity remain, causing withdrawal symptoms. It is characterized by vegetative symptomatology and also often accompanied by psychical disorders like anxiety, sleeping disorders and fleeting hallucinations. This study investigates olfaction impairment and potential changes during acute withdrawal syndrome (AWS). Olfactory measures were taken on Day3 and Day7 of abstinence among 23 heavy drinkers (HD) with a diagnose of alcohol addiction (F10.2) and 27 control subjects (CS) consuming one standard alcohol unit daily. The Sniffin' Sticks test battery was used for the assessment. Significant impairment of olfaction was identified in HD in discrimination and identification tests, against to CS in both testing days. It was also confirmed that psychopathological comorbidities (assessed by the SCL-90 test) in combination of alcohol abuse (dual diagnosis) influences the chemosensory ability. Noticeable deterioration shown the evidence of obsessive compulsive disease (OCD) and depression (D) in HDs. The results are statistically interpreted as a comparision of change in the frequency of scoring in boxplots for both testing group, at each olfactory test performed. Mann Whitney U test for distribution function comparision and Wilcoxon test for results pairing were used. The mere retreat of AWS in time, with the prolongation of the abstinence period does not indicate a change/improvement in olfactory functions. Also the medication locally standardized for the treatment of withdrawal symptoms, alterations in electrolyte equilibrium during AWS, the role of stress, impaired adaptation to the institutional treatment environment probably play the role in olfaction deterioration in long-term HDs in AWS.

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Olfactory bulb volume (OBV) is not associated with intensity of cortical activation

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Physiologically, higher OBV is related to increased olfactory identification performance. It is not clear whether an increased OBV relates to enhanced neural processing of olfactory information. In order to examine this relation, we conducted a fMRI study with 30 healthy and normosmic subjects in which we presented 2-phenylethanol with an expiration-triggered computer-controlled olfactometer. Analysis (on vs off contrasts, $p < 0.001$, uncorrected, cluster extent threshold: $k = 10$) revealed activation in the left inferior frontal gyrus ($k = 69$, $T = 4.35$), left temporal pole ($k = 57$, $T = 4.48$), right middle orbital gyrus ($k = 33$, $T = 4.33$), and right amygdala ($k = 11$, $T = 3.74$). Including OBV as a covariate in the analysis, only activation in the right hippocampus remained above threshold ($k = 19$, $T = 4.9$). This implies that OBV is not generally associated with intensity of olfactory cortical regions. This study was supported with funds from This Works Products Limited.

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The arousing properties of odors on facial perception – evidence from a dot-probe study

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The dot-probe paradigm has been seen as a gold standard of attentional bias towards emotional stimuli. In this paradigm, a neutral and emotional face pair is presented, the faces are removed and behind one of the faces is a probe. The task is to indicate on which side the probe appeared. Systematically faster responses to probes behind emotional faces are interpreted as that these receive more attention. Previous studies have shown that odors may enhance attention to congruent visual objects. However, some results in the literature indicate that odor might affect visual perception by arousal.

In this pre-registered study, the aim was to assess how odors influence attentional processes. We hypothesized either faster reaction times attributed to arousal or sustained attention to odor/face congruent pictures. We were also interested in time-on-task effects.

Using Bayesian linear models, we found strong evidence that emotional facial expressions were not attended in the dot-probe task or any general odor effects, even though the faces were rated as more arousing and emotional in odor contexts. Instead, we found that in the unpleasant odor condition response times decreased with time-on-task, whereas, in the no-odor and pleasant condition there was a slight increase in response times. In a separate study, we could show that affective odors are generally associated with congruent facial expressions.

Lately the dot-probe task has been questioned by several studies that have shown low reliability of the task. One hypothesis is that this type of covert orienting toward salient stimuli is very fast, so there is ample time for several attentional shifts before the appearance of the dot-probe. Thus, even though affective odors are associated with congruent facial expressions these might not facilitate sustained attention in the same manner as has been shown with congruent odors and visual objects. Time-on-task effects might explain inconsistent findings in the literature.

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The effect of olfactory stimulation on affective valence of dreams and affective state upon waking: results of a pilot study

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[Objectives] The focus of the presented project are effects of olfactory stimulation during sleep on affective valence of dreams and affective state upon waking. Effects of two "pure olfactory" stimuli, vanillin and thioglycolic acid, were investigated.

[Materials and methods] In weekly intervals, participants spent three nights in the sleep laboratory, to adapt to the research settings on the first one and receive olfactory stimulation (vanillin or thioglycolic acid) on the second or third one in a randomized design. On each night, nocturnal polysomnography (10 p.m. to circa 8 a.m.) was recorded and participants were woken up five minutes into the first REM phase that occurred after 4 a.m. Immediately after waking, they were asked to complete questionnaires on dream characteristics (e.g. pleasantness, presence of specific emotions and sensory modalities), affective state (core affect measure), and awareness of odor and its perceptual characteristics. They completed the same measures once again upon waking in the morning.

[Results] Findings in 60 participants show that along with the time of waking, there were significant small to medium effects of olfactory stimulation on certain aspects of participants' affective state upon waking and dream pleasantness. Namely, the greater the perceived odor pleasantness, the more interested, engaged, and optimistic the participant felt upon waking. Also, higher perceived odor intensity resulted in people's feeling more serene, calm, and relaxed upon waking. Odor familiarity positively affected reported dream pleasantness. This was regardless of the odorant used.

[Conclusions] There is a potential for odors to positively influence subjective dream pleasantness and modulate the affective state upon waking that is worth further exploration.

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Psychophysiological effects upon smelling essential oils and their compounds.

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There is a growing interest for using odor stimulations as an alternative to pharmacological treatment for patients suffering from anxiety. The influence of essential oil (EO) stimulations on the physiological response has been the topic of several studies and reviews. Identification of the emotion elicited upon an olfactory stimulation remains challenging. The advantage of using EOs instead of pure compounds has also still not been clearly addressed.

Would an EO perform systematically better than an isolated compound, and which compounds are the most effective within a given EO? This also raises the question of the synergistic effects in smelling compounds. A better understanding of these questions requires more studies using psychophysiology.

In the present study, the psychophysiological effect upon odor stimulation of 50 compounds (of which 10 were EOs) was assessed. 16 people were stimulated with different odorants and several physiological parameters were monitored: breathing frequency, heartbeat and skin conductance. Weak but significant physiological differences have been measured between smells. Two mixtures made up of the compounds present in some EOs reveal that the effect of concentration correlates with the physiological response. One mixture shows a decrease of the heart beat rate of ~4%, which is much more important than each isolated compound or EO taken alone.

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