ABSTRACTS

XXVIIth Annual Meeting of the European Chemoreception Research Organization, ECRO 2017

Wellcome Genome Campus Conference Centre, Cambridge, UK Organizer: Darren Logan

PLENARY LECTURES

PL1: Gastrophysics: On the art and science of pairing and sequencing flavours

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It is undoubtedly true that certain combinations of ingredients just taste better when sampled simultaneously, or in a particular sequence, than when sampled in a different temporal order. In this talk, I will highlight the importance of sequencing and pairing taste/flavour sensations, in the design of the meal itself, in the design of a mouthful of food, and when attempting to combine (or match) food and drink. I will take a look at the chemical, psychological, and computational approaches to combine flavours for maximal impact. I will evaluate three general principles of flavour matching: similarity - matching components based on common flavour compounds (or similar flavour profiles); contrast - combinations that are purposely chosen because they differ from each other (a strategy that is more common in the cuisine of some countries than others), and synergy (or emergence) – those combinations that together deliver new flavour experiences, or else harmonize with one another. I will argue that the psychological account (informed by an awareness of cultural differences), and to a lesser extent the chemical account, would appear to provide the most meaningful suggestions as far as effectively combining flavours is concerned.

Plenary 2: Genetics of appetitive behaviour: Can old dogs teach us new tricks?

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Genetic disruption of key neuronal pathways resulting in severe obesity have highlighted the role of the brain in the control of appetitive behaviour. I will briefly review the data from two well studied conditions within the genetics of obesity field, congenital melanocortin deficiency and Prader-Willi syndrome. I will then present new data that have emerged in recent years, that show us that these two 'old-dogs' still have the capacity to teach us new tricks.

Plenary 3: Olfactory evolution in drosophilids: from receptors to behaviours

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My group is interested in understanding the structure, function and evolution of neural circuits. We exploit the olfactory system of Drosophila melanogaster as a model, which is well-described, experimentally accessible and dynamically evolving. Furthermore, genomic and growing genetic access to closely related, but ecologically diverse, drosophilids provides an unparalleled foundation for comparative studies of their olfactory circuits. I will present our recent work on the evolution of the olfactory pathways of Drosophila sechellia, an island endemic that displays extreme specialisation for the Morinda citrifolia "noni" fruit as a food source and breeding site. We have started to define the molecular basis by which D. sechellia's olfactory receptors are re-tuned to the odours of its host and – through development of novel genetic tools in this species – how these different sensory pathways contribute to host-seeking behaviours.

Plenary 4: Nutritional and Flavor Programming

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There are sensitive periods during early life when mammals are particularly susceptible to environmental influences. In particular, the type and quality of the early diet is a modifiable determinant in the programming of later life health outcomes and risks for diseases. While much research focused on the nutrient quality of the diet, less attention was given to another important feature of early nutrition - its flavor. In this talk, I will review the evidence base from comparative studies that provided the foundation for the research on flavor programming in human infants. To characterize the sensitive period when hedonic responses to flavors are established, we conducted randomized clinical

trials that varied both the timing and duration of the experience. In general, one month of exposure before the age of four months was sufficient to shift the hedonic tone to a flavor experienced in either mother's milk or infant formula. The need for more research on the mechanisms underlying the development of sustainable food habits is significant since what the child eats determines in part who the child becomes.

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Plenary 5: Mechanisms of Adaptive Behavior across Moments and Millennia

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Animal behavior arises from an interplay between instinct and learning. Certain behaviors are innate and invariant across members of a species, suggesting they are genetically programmed into the nervous system. However, behavior must also be highly flexible to allow individuals to adapt to their unique and changing experience of the world. My lab uses a variety of functional and neural tracing techniques to define the architecture and algorithms of neural circuits in Drosophila, an insect that exhibits a rich repertoire of innate and learned behaviors mediated by a simple nervous system. I will describe recent work in which we explore how neural circuits in the fly brain can be adapted at different time scales: over evolutionary epochs to generate species-specific innate behaviors or over the course of an individual's lifetime to generate learned behavioral adaptations.

Plenary 6: The complex basis of bitterness perception

Wolfgang Meyerhof[1], Anja Voigt[1], Sandra Hübner[1], Kristina Loßow[1], Jonas Töle[1], Antje Stolzenburg[1,3], Anne Brockhoff[1], Kristina Blank[1], Ulrich Boehm[2], Maik Behrens[1]

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Bitter taste mediates innate rejection of potential toxins which limits intake of potentially harmful food. In mice and humans the countless bitter chemicals are recognized by oral chemoreceptor cells characterized by the expression of subsets of the available Tas2r bitter taste receptor gene repertoires providing broadly tuned but functionally dissimilar inputs. In mice the functional diversity of the peripheral input is propagated by afferent nerve fibers to 1st order gustatory neurons in the brain stem. Here as well as in other gustatory areas neurons are found that express bitter taste receptors. Blocking synaptic transmission by genetic manipulation in such neurons reduced behavioral avoidance of selected bitter chemicals. This finding suggests that the neural codes for some bitter compounds differ in the CNS and that mice could discriminate some bitter compounds. Indeed, we found that mice can be trained to distinguish arbutin from chlorhexidine and salicin from papaverine, pairs of compounds recognized by Tas2rs that are expressed in separate subpopulations of bitter sensing cells. We speculate that this complex molecular and cellular organization of bitter taste enables animals to appropriately deal with food containing potentially harmful substances.

SYMPOSIUM 1: SMELL, TASTE AND APPETITE IN NON-MODEL ORGANISMS

S1- Learning obesity biology from man's best friend.

Eleanor Raffan

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The global obesity epidemic has severe consequences for individual and population health. Obesity is highly heritable but, despite intensive efforts, only a small proportion of that heritability has been explained in humans. I exploit the unusual genetic architecture in dogs (a consequence of selective breeding) to map disease genes and provide new leads on obesity biology of relevance to all species. As an example, Labrador retrievers are commonly obese and very food-motivated. I will report my findings of a common mutation in Labradors associated with obesity and food motivation and show how we are extending that work to investigate the neural pathways regulating eating behaviour.

S2- Bitterness and Breeding: Bitter taste receptors in the domestic dog (Canis familiaris)

Matthew Gibbs[1], Alexander Horsfall[2], Ciaran O'Flynn[2], Neil Desforges[2], Oliver Forman[3], Marcel Winnig[4], Nicholas Holliday[5], Scott McGrane[2], Darren Logan[2]

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Bitter taste perception is well studied in humans but little is known about the extent to which bitter is perceived by the domestic dog (Canis familiaris). Studies in this species are of particular interest due to the many different dog breeds that have been derived by selective breeding. In this study we characterised the bitter taste receptor (TAS2R) repertoire of the domestic dog and compared the receptors ability to detect bitter compounds to their human orthologs. Dogs

have a reduced number of putatively functional bitter taste receptor genes (16) when compared to humans (25). In several cases the putatively functional dog gene is a pseudogene in humans. We found that generally dog T2Rs responded to the same compounds that activated their orthologous human receptors, but sensitivity between the species was often variable. Overall dogs have a set of TAS2R receptors that would appear to provide a broadly similar sense of bitter taste perception to that of humans. We also investigated the genomes of 103 different breeds of dog for putative functional mutations in the bitter taste receptors, identifying 68 mutations with the potential to influence receptor function. However for many breeds only a small number of individual dogs have been sequenced to date. Work is ongoing to determine which mutations are breed-specific and further in vitro characterisation of the receptors will show if the mutations have any true functional significance.

S3- Functional characterization of the ligand binding domain of the cat T1R1 taste receptor

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The umami taste receptor is a heterodimeric G-protein coupled receptor (GPCR), composed of two subunits called T1R1 and T1R3. Both subunits are class C GPCRs whose members share a common architecture composed of a large N-terminal domain (NTD) connected to a heptahelical transmembrane domain by a short cysteine-rich domain. Cellular assays combined with molecular docking and site-directed mutagenesis studies have revealed that the NTD of the T1R1 subunit contains the primary binding site for umami stimuli, such as L-glutamate (L-Glu) for humans. Inosine-5'-monophosphate (IMP) binds close to the opening of the NTD, responsible for the characteristic umami synergy between L-Glu and IMP. Functional cellular assays of the T1R1/T1R3 receptor have revealed species-dependent differences. Therefore, the human umami receptor responds specifically to L-Glu and L-Asp whereas mouse T1R1/T1R3 detects various amino acids. Because cats have a strictly carnivorous diet, they have strong preferences for amino acids. To better understand the structural basis of umami stimuli recognition, we measured the binding of selected amino acids to cat T1R1 (cT1R1). For this purpose, we expressed cT1R1-NTD in bacteria. Ligand binding quantified by intrinsic tryptophan fluorescence showed that cT1R1-NTD is capable of binding several L-amino acids with affinities in the micromolar range with enhancement activity of IMP. Our study demonstrates the feasibility to produce large amounts of recombinant cT1R1-NTD for structural studies.

S4- Dynamic evolution of olfactory receptor genes in mammals: Interaction between genomes and environments

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Diverse odor molecules in the environment are detected by olfactory receptors (ORs) expressed in the olfactory epithelium of the nasal cavity. There are ~400 and ~1,100 OR genes in the human and mouse genomes, respectively, constituting the largest multigene family in mammals. Thanks to the recent advancements in next-generation sequencing, the whole genome sequences of various non-model organisms have become available. Bioinformatic analyses using various genome sequences revealed that the numbers of OR genes vary greatly among species: African elephants have the largest number of functional OR genes ever examined, with ~2,000, while bottlenose dolphins, which have completely lost the olfactory apparatus, retain only ~10. By comparing the OR gene repertoires among organisms, we can infer how each species' olfactory world has evolved depending on its living environment.

As an excellent example showing a possible link between OR gene repertoires and ecology, we focus on primates. Primates are generally regarded as vision-oriented animals with low olfactory ability. However, the "vision-olfaction trade-off" hypothesis is too much simplified, because primates are highly diverse in morphology of the nose and eyes, activity patterns (diurnal or nocturnal), color vision systems, feeding habitats, etc. To reconstruct the entire view of the olfactory degeneration in primate evolution, we examined the OR gene repertoires from 24 diverse primate species. We traced the evolutionary fates of individual OR genes by identifying orthologous gene groups (OGGs), demonstrating that the rates of OR gene losses were accelerated at the ancestral branch of haplorhines and that of colobines, which occurred concomitantly with the acquisition of acute vision and the transition from frugivory to folivory, respectively. Interestingly, we also found independent accelerations of OR gene losses in an external branch to every hominoid species. Application of these analyses to other mammalian groups will be argued.

S5- Avian colour biases: an alternative method for assessing taste detection thresholds

Hannah Rowland

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Many species of animals, plants and micro-organisms employ defensive chemicals to reduce their likelihood of being ingested by their natural enemies. Understanding how bird predators assess the chemical content of warning coloured prey is relevant to our understanding of the adaptive significance of prey that have variable chemical defences, as well as avian taste perception. When domestic chicks experience a bitter tasting

chemical prior to encountering a warning coloured prey it enhances the chick's wariness towards those prey. Some chemicals seem to be intrinsically aversive to birds (e.g., quinine, anthranilic acid, 4-ketobenztriazine) but not all are aversive to a degree that they prevent ingestion. For example, quinine can produce attack biases by chickens against traits typically associated with aposematism, but denatonium benzoate does not. Chickens have only three bitter taste receptor genes in their genome: ggTas2r1, ggTas2r2 and ggTas2r7 for which a number of ligands have now been identified and tested in vitro. In this study I use a taste-cue colour-bias paradigm to examine chicken's responses to different concentrations of quinine, denatonium benzoate, quassia and caffeine. The assessment of unlearned responses to naturally occurring defensive chemicals may be an alternative method for assessing detection thresholds to the forced-choice solution-consumption method.

SYMPOSIUM 2: THE INTERFACES OF THE SENSES NUTRITION AND METABOLISM

S6- Interplay between appetite-stimulating and appetite-suppressing brain systems

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To maintain energy homeostasis, orexigenic (appetite-inducing) and anorexigenic (appetite suppressing) brain systems functionally interact to regulate food intake. Within the hypothalamus, neurons that express agouti-related protein (AgRP) sense orexigenic factors and orchestrate an increase in food-seeking behavior. In contrast, calcitonin gene-related peptide (CGRP)-expressing neurons in the parabrachial nucleus (PBN) suppress feeding. PBN CGRP neurons become active in response to anorexigenic hormones released following a meal, including amylin, secreted by the pancreas, and cholecystokinin (CCK), secreted by the small intestine. Additionally, exogenous compounds, such as lithium chloride (LiCl), a salt that creates gastric discomfort, and lipopolysaccharide (LPS), a bacterial cell wall component that induces inflammation, exert appetite-suppressing effects and activate PBN CGRP neurons. The effects of increasing the homeostatic drive to eat on feeding behavior during appetite suppressing conditions are unknown. Here, we show in mice that food deprivation or optogenetic activation of AgRP neurons induces feeding to overcome the appetite suppressing effects of amylin, CCK, and LiCl, but not LPS. AgRP neuron photostimulation can also increase feeding during chemogenetic-mediated stimulation of PBN CGRP neurons. AgRP neuron stimulation reduces Fos expression in PBN CGRP neurons across all conditions. Finally, stimulation of projections from AgRP neurons to the PBN increases feeding following administration of amylin, CCK, and LiCl, but not LPS. These results demonstrate that AgRP neurons are sufficient to increase feeding during non-inflammatory-based appetite suppression and to decrease activity in anorexigenic PBN CGRP neurons, thereby increasing food intake during homeostatic need.

S7- The interface between metabolic state and hypothalamic glucose sensing. Vanessa Routh

Vanessa Routh

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As the integrator of the neuroendocrine system, the hypothalamus carefully assesses the energy status of the body in order to appropriately partition resources to provide for each system without compromising the others. While doing so the hypothalamus must ensure that adequate glucose levels are preserved for brain function since glucose is the primary fuel of the brain. To this end, the hypothalamus contains specialized glucose sensing neurons which are scattered throughout the nuclei controlling distinct neuroendocrine functions. Glucose-excited (GE) neurons increase while glucose-inhibited (GI) neurons decrease their action potential frequency as extracellular brain glucose levels increase. We hypothesize that these neurons play a key role in enabling the hypothalamus to partition energy to meet these peripheral survival needs without endangering the brain's glucose supply. This lecture will first describe some of the varied mechanisms underlying glucose sensing in neurons in the hypothalamus. We will then focus on 2 populations of GI neurons with disparate functions in terms of energy partitioning: those in the ventromedial hypothalamus (VMH) and those in the lateral hypothalamus (LH). VMH GI neurons appear to be critical in generating the counterregulatory response to the insulin-induced hypoglycemia which occurs during insulin therapy in diabetes mellitus. In contrast, LH GI neurons, which express the peptide orexin, enhance excitatory drive onto downstream reward neurocircuitry. We have shown that their response to falling glucose levels is enhanced during energy deficit. While this would be beneficial during times of famine, it would also reinforce the drive for compensatory feeding after voluntary weight loss. These data support the hypothesis that altered glucose sensing in orexin neurons contributes to weight regain after dieting as well as binge-eating disorders by enhancing the reward value of food. The overall goal of this lecture is to discuss glucose sensing as a target of metabolic regulation which enables the body to maintain glucose levels under changing energy states. Supported in part by an AHA Grant in Aid (14GRNT20380639), JDRF 2-SRA-2014–269 and 3-SRA-2017-488-S-B and NIDDK R01 081538 and R01 103676.

S8- The effects of gastrointestinal protein sensing on gut hormone release and satiety

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Obesity is a global health issue. Understanding the physiological systems that sense macronutrients in order to regulate

appetite may allow us to exploit them to treat obesity. The gastrointestinal tract secretes a number of regulatory peptide hormones that regulate appetite and food intake, and coordinated changes in in circulating levels of these gut hormones partly mediate acute feelings of hunger and satiety.

High protein diets promote satiety and weight loss, but such intervention programmes are difficult to adhere to. The mechanisms by which protein suppresses appetite have not been conclusively established, but evidence suggests that the amino acids produced by protein digestion are sensed in the gut to modulate anorectic gut hormone release, and thus to regulate food intake.

Promiscuous amino acid sensing receptors, including the GPRC6a and the calcium sensing receptor (CaSR), are present on hormone-releasing enteroendocrine cells in the gastrointestinal tract. Specific amino acids can influence the release of appetite-modulating gut hormones, including Glucagon-Like Peptide-1 and Peptide YY, and suppress food intake in rodents and humans. We have identified the amino acids L-arginine, L-cysteine and L-phenylalanine as having powerful anorectic effects, and our data suggests that L-phenylalanine mediates its effects through the CaSR, which may thus represent a target for dietary or pharmacological approaches to appetite regulation. Hijacking gastrointestinal amino acid-sensing systems may therefore be a promising approach for the treatment of obesity.

S9- Reprogramming of Feeding Behavior by Diet

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While we understand how changes in the environment such as temperature and light direct animal behavior by acting acutely on neural circuits, we know less about the how the environment can lead to persistent changes in brain and behavior. Tackling this question has been challenging because it requires having a circuit-based understanding of the behavior and a mechanistic way to study how neural connections are changed by the environment. The reshaping of circuits that regulate food intake by a hyper-caloric diet in Drosophila melanogaster provides an attractive model for studying this question because the circuits are mapped, the behavior is easily quantifiable, and the environmental variables are simple to measure. We found that animals fed a Western style high-calorie diet show profound deregulation of feeding states: they incorrectly process the nutritional value of food, eat more, and become obese. We will present data showing how these behaviors are mediated by the metabolic-transcriptional reprogramming of distinct feeding circuits by diet and how their effect is persistent even after animals are returned to the control diet.

S10- Comparative transcriptomics pinpoints evolutionary driving forces of mammalian olfaction

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The importance of sensing the molecular environment is reflected in the genetic investment in encoding olfactory receptors (ORs), which constitute the largest mammalian gene family. The OR gene repertoire is largely species-specific, and shaped by the nature and necessity of chemosensory information for survival in each species' niche. In addition to differences in the ORs, the morphology, size, neural projections and organization of chemosensory epithelia vary remarkably across mammals, suggesting differences in wider gene expression networks. By combining RNA-seq with FACS in a hierarchical fashion from whole olfactory mucosa (WOM) to single olfactory sensory neurons (OSNs), we have identified the complete transcriptional profile of mouse OSNs, and their heterogeneity at the single cell level. But 25 years after the discovery of the ORs, the interspecific molecular heterogeneity of the olfactory system still remains largely unknown.

To study the evolutionary dynamics of gene expression in the olfactory system among species with different chemosensory niches, we performed RNA-seq of the WOM of six species of rodents, carnivores and primates (including humans). Our comparative transcriptome-wide analysis reveals a high degree of molecular conservation across 95 million years of mammalian evolution.

We found that ORs are expressed across a large dynamic range in these six species. RNA abundances correlate well with the number of OSNs expressing an OR. Combining RNA-seq with a phylogeny-based method that classifies ORs into orthologous gene groups (OGGs), we found that phylogenetic conservation does not imply conservation in OR gene expression: we find numerous examples of highly-expressed ORs specific for a single species or order. Our data further suggests that detection of food odors and semiochemicals are evolutionary driving forces of mammalian olfaction.

This experimental strategy has identified OR genes that may have been selected for different niches, and the possible driving evolutionary forces underlying OR gene expression distribution, thus contributing to a better understanding of the evolution of olfaction.

S11- Neuronal gluconeogenesis regulates systemic glucose homeostasis

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Gluconeogenesis, the synthesis of sugars from simple carbon molecules, is essential to maintain sugar homeostasis. We recently reported that a key gluconeogenic gene encoding Glucose-6-phosphatase (G6P) is mainly expressed in a few brain neurons of Drosophila melanogaster. Here we explore the function of G6P and gluconeogenesis in the fly brain. We show that G6P-GAL4 is expressed in several brain neurons including the two dorsomedial neuropeptide F (NPF) expressing neurons. Using the FRET-based glucose sensor pGlu700, we demonstrate that application of alanine to ex vivo brain preparations significantly increased intracellular glucose levels in G6P expressing neurons. Interestingly, G6P mutant flies develop hypoglycemia during starvation, a phenotype rescued by a G6P transgene expression. Remarkably, TrpA1-mediated activation of G6P-GAL4 expressing neurons rescued the hypoglycemia phenotype. These results suggest that neuronal gluconeogenesis leads the activation of a neural circuit that ultimately targets energy storage sites (fat body), which are essential for maintaining hemolymph sugar levels under starving conditions.

SYMPOSIUM 3: OXYGEN SENSING

S12- Acute Oxygen Sensing by Arterial Chemoreceptors

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Arterial chemoreceptors are essential for adaptation to environmental or pathological conditions presenting with hypoxemia. The main arterial chemoreceptor is the carotid body (CB), an organ located in the carotid bifurcation, which contains O_2-sensitive glomus cells with O_2-sensitive K^+ channels. Inhibition of these channels by hypoxia mediates depolarization and Ca^{2+}-dependent exocytotic transmitter release. These transmitters activate afferent sensory fibers acting on brainstem respiratory neurons to evoke hyperventilation. The mechanisms whereby changes in O_2 tension are translated into changes in K^+ channel activity have remained elusive. Studies performed on genetically modified animals have discarded the existence of several putative "O_2 sensors". These studies have also failed to support any role in acute O_2 sensing of an atypical olfactory receptor highly expressed in the CB. We have shown that ablation of the mouse Ndufs2 gene, which encodes a component of the ubiquinone-binding site in mitochondrial complex I (MCI), is absolutely necessary for mice or individual CB cells acute responsiveness to hypoxia. In addition, peripheral chemoreceptor cells possess a "signature metabolic profile" characterized by up-regulation of Hif2a, pyruvate carboxylase and several atypical mitochondrial subunits in combination with down-regulation of prolyl hydroxylase 3. These special metabolic features result in the accumulation of reduced ubiquinone (QH_2). Slow-down of the mitochondrial electron transport chain during hypoxia may further increase the QH_2 pool, thereby enhancing the production of reactive oxygen species and reduced pyridine nucleotides in MCI to signal membrane ion channels.

S13- Oxygen sensing by the mouse main olfactory epithelium

Frank Zufall

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This lecture will present neural strategies for the detection and avoidance of low environmental oxygen through the mouse olfactory system. Specifically, we will focus on a novel subpopulation of sensory neurons in the mouse main olfactory epithelium. These neurons, known as type B cells, express the soluble guanylate cyclase Gucy1b2 and the cation channel Trpc2. We have made significant progress in elucidating the role of these cells. Here, we will discuss the evidence that type B cells function as novel oxygen sensors in the main olfactory epithelium and that their activity drives aversive behavior. Our finding that Trpc2 is required in type B cells for the detection of low environmental oxygen has sparked renewed interest in its activation and function. We will discuss novel strategies for elucidating Trpc2 signaling mechanisms.

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S14- Deconstructing a switch in global animal state

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Animals adopt different global states in response to threats or opportunities. They do this by coordinated changes in neurochemistry that optimize their response to the situation at hand. Examples of global states include those evoked by a potential mate, or a predator. In the nematode C. elegans altered levels of ambient oxygen (O2) can evoke long-lasting changes in behaviour and physiology. I will delineate how molecular O2 sensors act in sensory receptors to encode O2 levels, how these sensors drive persistent changes in circuit activity, and how the activity of the O2 circuit reprograms other sensory responses. I will highlight how C. elegans stores a memory of its recent O2 environment and switches the valence of pheromone cues according to this memory. Finally, I will discuss how interleukin-17, a pro-inflammatory cytokine, acts like a neuromodulator, increasing the gain of the O2 sensing circuit and transforming the behavioural response to 21% O2 from one that is transient and dependent on additional coincident stimuli, to one that is unconditional and sustained.

SYMPOSIUM 4: PLACING THE PATIENT PERSPECTIVE AT THE HEART OF CHEMOSENSORY RESEARCH

S15- The patient perspective: the importance of engaging with clinicians and scientists

Tom Laughton

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For the patient, the impact of sensory loss can be devastating, impacting all areas of their lives and interactions with the world. Acknowledging this is a vital part of aiding the recovery or management of their condition.

Historically however, this has not been patients' experience, with little or no attention given to smell loss or its consequences.

In this talk, I will draw on my own and Fifth Sense members' histories to define the enormous benefits that engagement can bring.

S16- The clinical perspective: the diagnosis and treatment of clinical disorders.

Carl Philpott

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In 2010, Professor Philpott established the UK's first dedicated Smell and Taste Clinic at James Paget University Hospital. During this seminar he will describe his experience of managing patients with Smell and Taste disorders and discuss his role in leading clinical research into potential treatments. He will outline the deficiencies in the current evidence base for smell and taste disorders and their treatments.

S17- A role for chemosensory scientists in patient outreach, engagement and education.

Steven Munger

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The number of individuals with smell or taste impairments rivals that impacted by vision or hearing impairments. Even so, public advocacy and patient support related to chemosensory disorders lag far behind what is seen in the vision loss and hearing loss communities. The paucity of regular, organized and substantive interactions between those with anosmia, dysgeusia or other chemosensory disorders and the scientists that study the chemical senses creates a disconnect between patient needs and the focus of research and education efforts, and puts the entire community at a disadvantage in advocating for the importance of research funding to address this critical health area. In this talk, I will discuss ongoing efforts at the University of Florida Center for Smell and Taste – including our partnership with Fifth Sense on the recent SmellTaste2017 conference – to increase public education, patient engagement and community advocacy related to the chemical senses and chemosensory disorders.

S18- Gene Therapeutic Strategies for Congenital Anosmias: Peripheral and Central Restoration

Cedric Uytingco, Jeffrey Martens

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More than 200,000 patients each year report of chemosensory dysfunctions to their physicians, with olfactory dysfunction affecting at least 2.5 million individuals total in North America. While the etiologies of olfactory dysfunction are varied, a fraction of the cases can be attributed to congenital loss. One such cause is due to a class of pleiotropic disorders termed ciliopathies, which result from defective ciliary growth, maintenance, and/or function. The genetic basis of ciliopathies is remarkably complex, with an expanding list of more than 89 loci implicated in various disorders. Current treatment of ciliopathies and congenital olfactory dysfunctions are limited to symptomatic therapy with no long-term or curative solution. Here, we demonstrate the capacity of gene therapeutic restoration of ciliation in differentiated olfactory sensory neurons and odor detection in animal models of ciliopathies. More recently, gene therapeutic rescue of olfactory dysfunction has been extended to BBS knockout models of Bardet-Biedl Syndrome, which represents a treatable patient population. In these studies, we utilized adenoviral and clinically approved AAV vectors, which for the first time demonstrate gene transduction and rescue in the olfactory system. In addition, our new data expands on the capacity of gene therapy by preventing and restoring axonal convergence, olfactory bulb glomerular patterning, and odor-guided behaviors. Overall, these findings demonstrate the restorative capacity of the olfactory system and offers promise for the treatment of not only ciliopathies but also other congenital olfactory disorders.

SYMPOSIUM 5: OLFACTION IN EARLY DEVELOPMENT

S19- Responses of human neonates to odorants occurring in body secretions.

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The sense of smell is known to be functional in human neonates, enabling them to detect and discriminate odorants, and to memorize and adequately respond to odour cues and signals from their environment. During the last decades, responsiveness to milk and breast odours has been of

scientific interest due to their obvious ecological salience to newborns which is reflected in their positive responses to them. The more recent elucidation of odour-active compounds in milk and other bodily fluids makes it possible to not only study behavioural responses to the original odour mixtures but also to their constituents. The experiments presented here aimed to characterize the odour compositions of human colostrum and transitory milk, and neonatal responses to individually administered odorants. In view of their occurrence in body secretions, we hypothesized these odorants to elicit a positive behavioural response in the neonates.

Gas chromatography-olfactometry (GC-O) was applied to distillates from colostrum and transitory milk samples to identify the odorants which are most potent to the adult nose. Further, the detection and hedonic perception of odorants, known to occur in milk and sweat, were evaluated in 3-day-old neonates, as inferred from changes in facial expressions and respiratory rate.

GC-O analyses revealed that colostrum and transitory milk resemble each other in terms of their qualitative odorant compositions. The behavioural experiments indicated that neonates were more sensitive than adults to certain odorants. In contrast to our original hypothesis, however, the newborns predominantly evinced negative facial expressions, even for very low concentrations of the investigated odorants. These results will be discussed for their implications on our current understanding of neonatal odour learning and perception of odorants from body secretions.

S20- Behavioural responsiveness to single odorants and mixtures of odorants in the newborn rabbit.

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Olfaction is involved in vital behaviours such as interactions between mother and young, searching for food, predator avoidance, etc. Throughout life, these behaviours are displayed by animals (including humans) in the highly complex and changing olfactory environment composed by molecules - odorants - which are most often perceived as odours in the natural context of odour mixtures, after their processing by the olfactory system and brain. To deal with this complexity, organisms process odour mixtures in the elemental way (perception of one or several of the elements that compose the mixture) or weak/robust configural ways (perception of a configuration in addition to/at the expense of the elements). These modes of perception are shared between organisms across the animal kingdom. In the rabbit, newborns respond to a particular volatile component carried in the milk of lactating rabbit females, the mammary pheromone (MP; 2-methylbut-2-enal), which is perceived among more than 150 other components. The pheromone triggers the typical searching-oral seizing behaviour allowing for the pups to rapidly find and suck the nipples during the daily visit of the mother in the nest. The MP is also able to promote the very rapid acquisition of other odorants or mixtures of odorants by associative conditioning. Such odour learning can have direct consequences on the sucking success: after conditioning to an odorant by single association to the MP, pups become more efficient to get milk, the day after, when they are nursed by the mother previously scented with the conditioned stimulus (in comparison with a control odorant). This is also true if the mother is scented with a mixture that contains the learned odorant, except if the mixture is configurally perceived by the pups. Indeed, despite their relative immaturity, newborn rabbits are able to perceive more or less complex odour mixtures in the elemental or, depending on the mixtures (i.e., composition, ratio of components), configural way. This benefits experimental studies investigating the sensory and cognitive abilities that function at birth in this species and are involved in early adaptation to the environment.

S21- Parent-offspring recognition in a songbird: How important is olfaction?

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The mechanisms underlying parent-offspring recognition have fascinated researchers for centuries. However, the ability of avian chicks to recognize parental odours at hatching has been completely neglected, despite evidence that birds employ social chemosignals. We previously developed a simple, in-the-hand testing paradigm that uses the duration of stereotyped begging behaviour to quantify odour discrimination in day-old Zebra Finches (Taeniopygia guttata). Here we used the same paradigm to investigate whether Zebra Finch chicks (Taeniopygia guttata) could identify odours of their parents. In the first experiment, chicks begged significantly longer in response to the odour of their genetic mother or father compared to the odour of an unfamiliar adult of the same sex and reproductive status. In a second experiment, we cross-fostered eggs and tested the response of hatchlings to the scent of a genetic vs. foster parent. Chicks from cross-fostered eggs responded significantly more to the odour of their genetic mother than their foster mother, but did not discriminate between odours of their genetic or foster fathers. Our results suggest: (1) Zebra Finch chicks are capable of recognizing odour cues of their genetic parents immediately after hatching, and (2) Chicks retained the ability to recognize their genetic mothers, even after they were cross-fostered and incubated by unrelated adults.

S22- Zebrafish kin recognition is mediated via a newly described teleostean accessory olfactory system.

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Zebrafish imprint on visual (at day 5 post fertilization) and olfactory (at day 6 post fertilization) cues coming from kin siblings. Here, zebrafish larvae were raised experimentally in order to generate imprinted and non-imprinted specimens. Stimulation tests (at day 9) using kin odor show a specific increase of neuronal activity (shown with pERK) in crypt cells and in the mediodorsal olfactory bulb only in imprinted larvae, but not in non-imprinted larvae, suggesting that imprinting triggers neural changes at the olfactory epithelium level and the central nervous system. Additional tracing experiments in adult zebrafish show an associated accessory olfactory pathway originating from crypt and microvillous olfactory sensory cells running via mediodorsal olfactory bub and medial amygdala to the tuberal hypothalamus, which is diagnostic in synaptic succession of the vomeronasal system seen in land vertebrates.

S23- Ontogeny of nestmate recognition in *Polistes* paper wasps

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Prenatal and early olfactory learning trigger significant effects on development in general, and exquisitely on behaviour, in a wide variety of organisms. Early learning has been largely investigated in vertebrates, but the process can occur also in invertebrates. In social insects early learning appears to influence important features of social life, such as nestmate recognition. Social insects can discriminate between nestmates and aliens by comparing the chemical phenotype of an individual with the neural representation of their own colony odor (template). Yet, relatively little is known about the ontogeny of nestmate recognition, and the learning processes that might be involved. Individuals could acquire the information to create their template from their social environment or through self-referent phenotype matching, which requires no learning of environmental kinship cues. The acquisition process might be restricted to an early sensitive period, or the template could be updated during adult life according to social requirements. Here, we present the results of our studies on the ontogeny of nestmate recognition in Polistes paper wasps, a model genus for the study of recognition. Through differential odour experience experiments carried out at different developmental stages, we showed that workers of Polistes dominula appear to develop their nestmate recognition abilities based on their social context rather than through prenatal and early learning or self-matching. Interestingly, our results showed that wasps do not form their referent template during the pupal or early adult phase simply from the nest paper and they are not able to acquire kinship information through self-matching. Our studies suggest that the social context provided by the nest shapes recognition abilities in Polistes wasps, therefore shedding new light on the ontogeny of nestmate recognition in paper wasps and in other social insects.

SYMPOSIUM 6: CHEMICAL SPACE OF CHEMOSENSORY COMPOUNDS

S24- The bitter chemical space: properties, predictors and relation to toxicity

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The bitter taste sensation is typically considered to signal toxicity and is elicited by molecules of widely varying chemical structure, summarized in BitterDB http://bitterdb.agri.huji.ac.il. The relation between chemical structure and bitterness, and between taste and bitterness can now be explored. In particular, we characterize the chemical properties of bitter compounds, develop bitterness predictor, and quantify the relation between toxicity and bitterness.

Our decision trees-based machine learning tool BitterPredict correctly classifies 80% of the compounds in the hold-out test set and in three independent external sets, into bitter and non-bitter compounds. The total charge, the hydrophobic component of the saturated carbons and descriptors of molecular surface are the most important contributors to the classifier. To estimate the bitterness-toxicity paradigm, we analyzed large repositories of toxic compounds. Only 40% of the toxic compounds (as well as of random compounds or compounds from food) are either known or predicted to be bitter. In comparison, ~70% of FDA-approved drugs are predicted to be bitter. Over half of the bitter compounds have some toxicity. The distribution of oral LD_{50} values of bitter compounds is skewed to less lethal values compared to that of a library of oral poisons, and is similar to the LD_{50} distribution of non-bitter compounds. Furthermore, no trend linking LD_{50} values with the number of activated bitter taste receptors (TAS2Rs) subtypes is apparent in the currently available data.

Thus, using BitterDB and BitterPredict we show that the bitterness-toxicity overlap is partial, supporting the idea that activation of bitter taste receptors has physiological roles beyond alerting against poisons, in agreement with the extra-oral expression of bitter taste receptors.

"*Bitter or not? BitterPredict, a tool for predicting taste from chemical structure*" Dagan-Wiener et al, submitted
"*The taste of toxicity: a quantitative analysis of bitter and toxic compounds*" Nissim, Dagan-Wiener and Niv, submitted

S25- Predicting Perception from Molecular Structure: From Molecules to Mixtures

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A fundamental problem in neuroscience is mapping the physical properties of a stimulus to perceptual characteristics. In vision, wavelength translates into color; in audition, frequency translates into pitch. By contrast, the mapping from chemical structure to olfactory percept is poorly understood. Recent progress in the field has begun to address this gap in the field, and here we will review several emerging models. First, we developed a model that can distinguish odorous from odorless molecules based on their physicochemical properties with 72% accuracy (AUC = 0.82) in external validation. Second, we participated in the DREAM Olfaction Prediction Challenge, which predicted the intensity ($r = 0.78$, $p < 10^{-228}$), pleasantness ($r = 0.71$, $p < 10^{-8}$), and quality ($r = 0.55$, $p < 10^{-5}$) of single molecules in external validation, closely approaching the test-retest correlations for these same properties. Third, we developed a model that allows us to use receptor responses to single odors to predict receptor responses to complex mixtures responses, which is a powerful method to extrapolate from small data sets to a large variety of complex mixtures. Charles Sell, in 2006, predicted that "...our ability to predict odor properties of molecules will not improve significantly in the near future." Here we argue that the field has now advanced to the point where progress is limited more by data collection than by modeling techniques.

S26- Data mining in odorant space: The quest for better chemical feature descriptors

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Finding new ligands for olfactory receptors is like searching the proverbial needle in the haystack. Huge databases of chemicals are available that contain thousands or even millions of compounds. But in the domain of olfaction, it is often impractical to screen such huge numbers of compounds for their activity at a specific olfactory receptor, e.g. in cellular assays. Even more so, evaluating scent by human experts is often restricted to a few dozens of odorants. Thus, there is a need for computer-assisted methods that help to narrow down that huge search space to manageable numbers of compounds.

"Virtual screening" uses data mining and machine learning methods to search huge chemical databases for odorants that are chemically similar to known ligands for a specific receptors, or to other compounds with a desired scent profile. The first step in virtual screening is to encode odorants using physical chemical properties, representing them as points in a high-dimensional space.

The choice of chemical descriptors is crucial for successful virtual screening, because even the most advanced machine learning method can only succeed if the descriptors appropriately represent those chemical features that are relevant for receptor-ligand interaction.

Virtual screening has been used with great success in drug discovery for over two decades. Most chemical descriptors that are available in software packages today have been developed with drug-like molecules in mind. However, the chemistry of odorants differs quite substantially from drug-like compounds.

Therefore, the question arises whether those descriptors actually capture the chemical features that most effectively describe odorant space?

Starting from our efforts to deorphanise the MOR18-2 olfactory receptor, we carried out an iterated virtual/biological screening campaign to comprehensively probe its molecular receptive range. Using the measured activities of 214 odorants, we built retrospective models that predict the response of MOR18-2 from the chemical structure of any odorant. We benchmarked multiple sets of available descriptors for their ability to capture essential chemical properties governing ligand affinity. In addition, we evaluated the predictive performance of a descriptor based on the frequencies of vibrational normal modes. Notably, we found that best predictive performance was achieved when combining "conventional" with vibrational descriptors.

Our results indicate that virtual screening in olfaction might currently be hampered by a lack of suitable descriptors. Furthermore, our findings point out routes towards better chemical descriptors that might improve virtual screening in olfaction, and provide better insight into structure-activity relationships for olfactory receptors.

S27- Systematic structure-odour-activity relationship exploration - towards characterising inter-individual differences in odour perception

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Odor character and odor impact are important aspects in the perception of aromas and smells, be it in foods or fragrance applications, or in smells in our everyday surrounding. While aroma compounds are mostly resolved nowadays, little is known about smells emanating from our artificial world such as plastic products and other non-food materials. Moreover, metabolic derivatives of commonly known odorants, either as side-products in plants or being formed in metabolic processes e.g. within the nose, are likewise barely explored, mostly due to the fact that those substances are commonly not commercially available. Moreover, odorants that remained unresolved until today may be exceptionally

challenging with regards to their chemical characterization as they might have been elapsed detection due to their extremely low odor thresholds and, accordingly, low concentrations in nature or modern products. We are faced with this problem on a daily basis when resolving unknown molecular structures of potent odorants. Moreover, we are often confronted wih contradictory sensory reports, i.e. some individuals being completely unable to perceive specific smells while others are highly perceptible or even disturbed by specific smell impressions. To address this problem, we apply the strategy of systematically exploring structure-odour activity relationships encompassing comprehensive synthetic-analytical strategies, together with human-sensory evaluation of the respective synthesized compounds. In this respect, we specifically aim at understanding how these substances are perceived by different individuals; thereby, it often turns out that odorants do not only elicit divergent smell impressions but may be also perceived with major differences in their smell intensity. These phenomena will be addressed in the lecture with the examples of structurally related terpenoid and aromatic substances, and potent fatty acid-derived odorants [1–5].

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SYMPOSIUM 7: ODOR ENCODING IN THE BRAIN

S28- Interhemispheric connections between olfactory bulbs improve odor detection

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In most sensory systems, bilateral arrangement of the pair of sensory organs is important for detecting the stimulus direction and location. The interhemispheric inhibitory interactions that use contralateral information from these two separate sensory channels are widely agreed to be important for generating delay lines across sensory organs. In most of these models, the delay between the arrival of sensory stimulus to the bilaterally arranged sensory organs are suggested to be utilized to suppress the signal coming from the contralateral sensory organ via contralateral inhibition. Such contralateral inhibition is therefore proposed to be important to increase the relative strength of the signal on the ipsilateral side, where the sensory stimulus is located, which facilitates the localization of the stimulus source.

Using anatomical tracing and functional brain imaging in adult zebrafish, we showed that two olfactory bulbs exhibit both inhibitory and excitatory connections. We showed that the inhibitory interhemispheric connections are established via diffuse projections of the olfactory cortices onto contralateral olfactory bulb interneurons. Moreover, we showed that mitral cells send spatially organized direct excitatory connections to their functionally identical sisters in the contralateral olfactory bulbs. Our results suggest that this glomeruli specific direct contralateral excitation can be recruited even by relatively weak olfactory stimuli and it is more sensitive than the polysynaptic contralateral inhibition. Finally, we showed that the combination of spatially organized contralateral excitation together with the spatially diffuse contralateral inhibition, improves the detection of specific odor signals against a broad background odor, by broadly subtracting background noise while compensating for the intra-bulbar mixture suppression. We propose that such interhemispheric connections are not only useful for localizing stimulus sources, but also provide a novel mechanism that can improve the detection of odors activating only few glomeruli, such as reproductive pheromones, social or alarm cues.

S29- Perception and encoding of temporally patterned odor stimuli in the mouse olfactory bulb

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Intensity fluctuations inherent to natural odor plumes are strongly modulated by the turbulent airflows in which they are carried. Odor plumes therefore hold a rich temporal structure of odor concentration variation which contains information about the location and nature of an odor source. It has been hypothesized that animals perceive and process these odor intensity fluctuations allowing for odor scene segmentation when navigating through natural environments. We developed a high-speed odor delivery device capable of replicating many temporal features recorded from naturally occurring odor plumes. Reliably controlling the temporal profile of odor stimuli enables us to investigate the psychophysical limits for discriminating odor correlation structure in an automated behavioral system. The high-throughput nature of these experiments provided us with an appropriate stimulus range for

performing targeted multi-photon Ca^{2+} imaging experiments in olfactory bulb projection neurons to elucidate potential frequency tuning properties and encoding mechanisms. Behavioral experiments revealed a perceptual threshold frequency for odor correlation structure well over sniff rate (>15Hz). Mice trained to discriminate between the same odor presented at different frequencies showed a lower perceptual threshold (~8Hz). Recording Ca^{2+} responses in anaesthetized animals to odors fluctuating at frequencies between 2-40Hz revealed different response kinetics in a subset of mitral and tufted cells indicating some level of temporal sensitivity. We conclude that odor concentration fluctuations are encoded in the mouse olfactory bulb at sub-sniff resolution, and that perception of their temporal features may aid in odor scene segmentation.

SYMPOSIUM 8: LOST IN TRANSDUCTION: OLFACTORY SIGNALLING ACROSS PHYLA

S30- Asymmetric ephaptic neuronal interaction in an olfactory circuit

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Olfactory receptor neurons (ORNs) housed in the same sensory hair in Drosophila interact functionally with one another by means of direct electrical interaction, known as ephaptic coupling. Despite its ubiquity, how ephaptic communication regulates olfactory function is poorly understood. In a systematic electrophysiological survey, we show that ephaptic interaction between adjacent neighboring ORNs is asymmetric across multiple neuronal types. Furthermore, using genetically encoded marker in combination with serial block-face electron microscopy (SBEM) and 3D reconstruction imaging technologies, we find that the morphological features of neighboring ORNs are also asymmetric and the physically larger ORN in a pair exerts stronger ephaptic interactions upon its neighbor. Our study reveals the common functional characteristics and biophysical principles of a novel form of olfactory circuit interaction mechanism. These findings have the potential to bring novel insights into our understanding of olfactory information processing across insect species.

S31- Olfactory network formation and function in the amphibian Xenopus laevis

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In contrast to the single sensory surface present in teleost fishes, the mammalian olfactory system is defined by spatially segregated subsystems with distinct molecular and functional characteristics, chief among them the main olfactory epithelium and the vomeronasal organ. Most amphibians, including Xenopus laevis, also have anatomically segregated main and vomeronasal olfactory systems, but at the cellular and molecular level the segregation differs from that found in mammals. The general structure and organization of the Xenopus olfactory network is also quite different from that found in other vertebrates. We analyzed the olfactory system of the secondarily aquatic pipid frog Xenopus laevis, and report the existence of two odor-processing streams, sharply segregated in the main olfactory bulb and partially segregated in the main olfactory epithelium of premetamorphic larvae. The two streams consist of morphologically different receptor neuron types and exhibit diverse transduction pathways and different odorant sensitivity. We have characterized expression patterns of four olfactory receptor gene families, and concomitantly have analyzed odor-evoked neuronal activity in the olfactory epithelium and the olfactory bulb. We found that axons of single receptor neurons bifurcate and mostly innervate more than one glomerulus, challenging the predominant opinion about olfactory wiring. During metamorphosis, extensive cell death and mitosis events lead to a remodeling of the larval main olfactory epithelium (detecting aquatic odors) into the adult 'air nose', with concomitant formation of a novel olfactory organ, the so-called 'water nose'. We found that both olfactory receptor expression patterns and corresponding odor responses faithfully relocate from larval main olfactory epithelium fo adult water nose.

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S32- Signaling Mechanisms in the Mouse Accessory Olfactory System

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In most mammals, conspecific chemical communication controls complex behaviors. Information about individuality, social and reproductive status is conveyed by an elusive class of chemical cues – pheromones. The highly reproducible character of pheromone responses offers a unique opportunity to uncover the neuronal basis of genetically

programmed behavior. The accessory olfactory system is a key component in rodent conspecific chemical communication. However, sensory detection and coding of socially relevant chemosignals within the vomeronasal organ and downstream brain areas - the accessory olfactory bulb, the 'vomeronasal' amygdala and the hypothalamus - is poorly understood. Combining molecular, biochemical, (electro)physiological, and live-cell imaging methods, as well as behavioral techniques in wildtype and mutant mouse models, we have extended existing models of sensory signal transduction in the vomeronasal organ and analyzed aspects underlying the principle coding logic of pheromone detection. More recently, we have begun to address specific signaling strategies implemented in the rodent accessory olfactory bulb. Both in and ex vivo approaches from different physiological angles reveal novel aspects of chemosensory signaling mechanisms in the mouse accessory olfactory system.

S33- Mammalian olfactory signal transduction: an update

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Olfactory signal transduction is the series of events for the transformation of a chemical signal, an odorant, in an electric one that is then sent to the brain. The very first step consists of the binding of odorant molecules to olfactory receptors, located on the cilia of olfactory sensory neurons (OSNs). This triggers an enzymatic cascade that leads to the increase of intraciliary cAMP followed by ion channels opening: first, directly gated by cAMP, the cyclic nucleotide gated (CNG) channels and then the calcium activated chloride channels (CaCC).

By the mid 90' the picture was almost complete. Indeed, all the elements involved in olfactory transduction were known, bar one: the identity of the CaCC. Although the Cl^- current has largely been characterized biophysically, the molecular identity and physiological role of the CaCC were largely elusive. Despite several attempts, the olfactory CaCC identity has been a mystery till the discovery (in other cell types) of a new family of chloride channels: the TMEM16. In 2009 it was demonstrated that one member of this family, TMEM16b, was indeed the olfactory native channel. Surprisingly, it was shown that TMEM16B has a limited to possibly no role in olfactory physiology and behavior.

What is the role of TMEM16B, then? We decided to re-examine the question and found that mice lacking TMEM16B have an impaired odor-guided food finding behavior and their OSNs have altered spontaneous and also evoked action potential firing.

In summary, the overall understanding of olfactory signal transduction needs to be updated to include the functions for the long time unknown and elusive calcium activated chloride channel, TMEM16B.

SYMPOSIUM 9: ACTIVE SAMPLING AND ENCODING OF DYNAMIC ODOR CUES

S34 – Abstract withdrawn as presenter did not attend AQ1

S35- Active sampling and encoding of aquatic odors by motile-cilia mediated flow in the nose

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Motile cilia are actively beating hair-like structures that cover the surface of multiple epithelia. The flow that ciliary beating generates is utilized for diverse functions and depends on the spatial location and biophysical properties of cilia. Motile cilia in the nose of aquatic vertebrates are spatially organized and stably beat with an asymmetric pattern, resulting in a robust and stereotypical flow around the nose. Our results demonstrate that these flow fields attract odors to the nose pit and facilitate detection of odors by the olfactory system in stagnant environments. Moreover, we show that ciliary beating quickly exchanges the content of the nose, thereby improving the temporal resolution of the olfactory system for detecting dynamic changes of odor plumes in turbulent environments. Altogether, our work unravels a central function of ciliary beating for generating flow fields that increase the sensitivity and the temporal resolution of olfactory computations in the vertebrate brain.

S36- Adaptive active sampling behaviour underlies dynamic contextual modulation in the olfactory bulb

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The ability to respond to sensory stimuli according to learning and context is vital for orchestrating appropriate behaviour. Activity already in early sensory cortices is modulated by contextual information e.g. locomotion, attention and exploratory behaviour. The olfactory bulb (OB) is the very first site of odor information processing, yet already modulation of OB neural output by a wealth of contexts and behavioural tasks has been described; the mechanistic and functional understanding of this, however, is limited. Here, using intracellular recordings from the OB in behaving mice, we show that odour-evoked activity in identified principal neurons dramatically changes even during rapid olfactory learning episodes, increasing excitatory representation of odours, and enhancing stimulus detectability. Increased excitation was always accompanied by increases in rapid sniffing behaviour. Rapid switching between engaged task behavior and passive exposure dynamically modulated the sniffing

strategy, resulting in profound and reversible changes in excitatory odour responses. Evoking sniff changes in awake or anaesthetised mice caused similar but weaker response changes, showing that the influence of sniffing on response is dependent on behavior. Thus, context-dependent adjustments in active sampling strategies can directly impact on early sensory responses, improving stimulus representation to facilitate behavior.

S37- Temporal precision in insect olfaction

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Insects can segregate target odors from background odors based on few millisecond differences in the arrival of odorants. This high olfactory processing speed poses temporal constraints on the neural code for odors. The prevailing model of insect olfaction assumes that odors are encoded in form of spike rate difference across receptor neurons. However, the limited temporal resolution of a rate code is not compatible with the rapid olfactory processing observed in insects. Using Drosophila as a model, we tested whether its olfactory system could use spike timing to encode properties of odorant stimuli which cannot be captured by rate coding. I will show that free flying flies use short asynchronies between two overlapping odorant streams for odor-background segregation; and I will show that odorant-induced spikes in sensory neurons occur with sub-millisecond precision. These data suggest that the insect olfactory system uses spike timing, rather than spike rates, across sensory neurons to encode the identity of rapidly fluctuating odorants as they occur in natural odor plumes.

STUDENT & YOUNG SCIENTIST SYMPOSIUM

Y1- The Receptor Repertoires Behind Varying Odorant Concentrations

Xiaoyang Serene Hu[1], Kentaro Ikegami[1,3], Marcelo Zapata[1], Natasha Vaidya[1,4], Matthew Do[1], Claire de March[1], Hiroaki Matsunami[1,2]

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How odor identity and intensity is encoded by neurons remains a fundamental problem in olfaction. One important question that remains unanswered is how the mammalian olfactory system is organized at the receptor level to discriminate between odorant concentrations that alter odor intensity and sometimes, odor quality. In this study, we provide a comprehensive analysis of how single odorants across a wide concentration range is differentially encoded by the mice odorant receptor repertoire. Using a combination of in vivo, in situ and in silico methods we examined the changes in OR activation patterns across 10,000-fold concentration ranges for acetophenone and 2,5-dihydro-2,4,5-trimethylthiazoline (TMT). To obtain this large dataset, we utilized our recently developed in vivo method, involving RNA capture with phosphorylated ribosomal protein S6 (pS6), a neuronal activation marker, combined with next generation RNA sequencing. To improve the sensitivity of our gene enrichment analysis, we further refined the gene definitions of mouse ORs. Additionally, we adopted a new mapping method which allows for the rapid quantification of OR abundance and successfully identified ORs activated at 100-fold lower odorant concentrations than before. This enhanced method offers certain advantages over existing deorphanization strategies in terms of greater detection sensitivity and significant reduction in processing time. Consistently, we found that the number of responsive ORs gradually decrease when we lower the odorant concentrations from 100% to 0.01% for both odorants. We subsequently used double label fluorescent in situ hybridization against individual ORs and immunohistochemistry against pS6 to confirm that each OR exhibits distinct activation patterns across odorant concentrations. The cognate ORs can be classified into three broad categories (1) ORs activated only at higher concentrations, (2) ORs which remain activated consistently, across a wide concentration range, and (3) ORs which are preferentially activated at certain ranges of odorant concentrations. Finally, we generated the three-dimensional structure of a high-affinity OR for acetophenone by homology modeling, and obtained favorable free energies through the receptor's interactions with the ligand. This study allows for the identification of ORs that are highly responsive towards a given odorant, and sheds light on the logic by which unique OR repertoires may collectively allow for the discrimination of varying concentrations of odorant.

Y2- Connectomic analysis of third order olfactory neurons in Drosophila

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Neural representations of the chemosensory world generate both learned and instinctive behaviours. Olfactory systems detect a huge range of volatile by combining patterns of activity across input channels. Each can detect a range of odorants such so that even the simple vinegar fly, Drosophila

melanogaster, can classify odorants never before encountered. While most odorants only elicit a strong behavioural response after associative learning, ecologically meaningful and evolutionarily significant odour channels trigger innate behavioural paradigms likely through hard-wired, genetically and developmentally pre-programmed circuits.

Many of the key molecular and computational principles behind the shallower processing layers in the olfactory system have been established in a variety of insect models. But the identity and functioning of third order neurons, particularly those outside the mushroom body, the centre for associative learning, are poorly understood. How these neurons are organised to produce innate responses and how this representation may interact with learning and memory remain open questions.

Whole brain electron microscopy volumes for both larval and adult D. melanogaster brains provide the opportunity to understand the organisation of an innate olfactory processing centre in the insect brain, the lateral horn, at synaptic resolution. We present connectomic findings describing a) the physical organisation of second order olfactory inputs into the adult lateral horn b) a class of adult lateral horn neuron that integrates information from both second order odour channels and memory read-out neurons c) newly discovered lateral horn neurons that innervate the associative learning centre d) third order local neurons of the adult lateral horn that integrate across sensory modalities. We compare these findings in the adult fruit fly to the larva, in which ten-fold fewer lateral horn neurons must accomplish comparable tasks: ascribing some of the same odour channels with similar innate valence, integrating them with other modalities and the outcome of prior learning. Our observations provide a direct circuit mechanism for learned and innate olfactory representations to interact, likely recruiting the same downstream circuitry to generate appropriate behaviour.

Y3- Autonomic response in newborns to relatively strong and mild trigeminal odorants

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Human newborns do not have a fixed set of olfactory likes or dislikes because they acquire them mainly by evaluative conditioning. However, odorants elicit differential preferential responses, even without prior experience. Odorant stimuli generally convey olfaction per se as well as some degree of intranasal chemesthesis. This tactile confound of the odour sensation mediated by the trigeminal system, whose stimulation may result into neurological airway protection processes, could be a potential predictor of spontaneous preferential responsiveness to unknown odorants. In our study we explored if unfamiliar odorants with contrasted trigeminal intensity led to stronger defensive response (heart rate (HR) acceleration) indicative of arousal magnitude and perceived unpleasantness in newborns. To fifty of them (two-to-three-days old) we administered three randomized order odorants; two of them unfamiliar with different trigeminal pungency (one strong, one weak) and one blank. Repeated-measures ANOVA revealed that the newborns' HR exhibited a significant interaction of the odorant with the course of HR variation in the first trial. Further, repeated planned contrasts showed a significant difference between odorants across repeated measures circa half a minute after the presentation. However, paired samples t-tests have shown that the significant difference was for the blank stimulus only, the strong trigeminal odorants induced a slight HR increase compared to which the weak ones induced a stronger HR increase; blank stimuli a marked drop. Further, no such effects were found in the two, consequent trials. At this moment, our findings do not support asymmetric processing of odours with different trigeminal component at the HR level. However, we do provide further evidence that newborns in irregular sleep respond differentially to very low dilutions of odorants.

Y4- Insulin-dependent maturation of newly generated olfactory sensory neurons after injury

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Insulin is a peptide hormone that regulates glucose metabolism, but also has different regulatory roles as an extrinsic signals in the brain. However, it is poorly understood whether insulin signaling affects ongoing incorporation of newly generated olfactory sensory neurons (OSNs) to maintain homeostasis in the olfactory epithelium (OE). Here, we examined whether insulin signaling affects the dynamic incorporation of new OSNs in adult mice after injury. Mice were administered Streptozotocin (STZ) to ablate pancreatic β cells, resulting in hypoinsulinemia. Methimazole, an olfactotoxicity-inducing drug, was also intraperitoneally injected to ablate OSNs in the STZ-administered mice. Up to 7 days post-injury, there was no difference in the numbers of recovering mature and apoptotic OSNs between the STZ-administered and control

(saline-administered) mice. However, between days 7 and 28, the STZ-administered mice showed remarkably fewer mature OSNs and more apoptotic OSNs than control mice. By day 28, control OE was restored to its pre-injury condition, while STZ-treated mice still had OEs that had not recovered. Consistent with fewer mature OSNs in the STZ-administered mice, electroolfactogram responses induced by odorants at day 28 following injury were significantly reduced compared with those in control mice. Furthermore, replenishment of insulin in STZ-administered mice during days 7 to 14 post-injury promoted the recovery of the OE. These results indicate that insulin signaling is involved in homeostatic regeneration of the OE following injury, and that newly generated OSNs have a high susceptibility to insulin signaling for their maturation following day 7 post-injury.

Y5- Dynamics in insect olfactory receptors lead to qualitatively different responses to odour mixtures

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Insects typically encounter complex mixtures of odorants in their natural environment. We therefore asked whether and how their responses to odorant mixtures and single components differ in the olfactory receptors, and the input and output neurons of the first olfactory processing center, the antennal lobe. To approach this question, we built a statistical model of the full receptor repertoire of honey bees and modelled the biophysical processes of receptor binding and activation by a set of ordinary differential equations. Using simulations and mathematical analysis of the equations, we found that dynamics in olfactory receptors induce statistically different responses to mixtures and to single components in receptor neurons, namely, the response latency of olfactory receptor neurons decreases and their response patterns become less variable across concentrations with increasing number of chemical components in the mixture. We confirmed the model's predictions for response latencies by single sensillum recordings in Drosophila. We next built a simple model of honey bees' antennal lobe network and proposed that these mixture effects are preserved in the output neurons of the antennal lobe. Our results suggest that the insect olfactory system encodes mixtures more efficiently than single odorants, which resonates well with the observation that chemical signaling in nature predominantly utilizes mixtures.

Y6- The chemical basis of individual recognition in the domestic cat (*Felis silvestris catus*)

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Domestic cats spray their urine onto a vertical surface for territorial marks. Their urine emit several volatile compounds some of which may be scent signals. Our previous studies demonstrated that derivatives of the urinary amino acid known as felinine are scent signals for species, sex, and age recognitions in cats. However, little is known which urinary compounds are used for individual recognition in cats. The aim of this study was to understand the chemical basis of individual recognition in cats. To identify key compounds for individual recognition in cats, we focused on the functional behavior called as flehmen response; cats raise their head and hold their mouth partially open for a few second. It is known that the flehmen response is observed in male cats after sniffing estrous female urine. In addition, we found that cats exhibited the flehmen response toward unfamiliar urine, but not familiar urine, suggesting that flehmen bioactive compounds are candidates of key compounds for individual recognition in this species. Therefore, flehmen bioactive compounds were isolated from cat urine using liquid chromatography. Our analyses showed that cats do the flehmen response toward the fraction containing urinary fatty acids. GC-MS analyses showed that chemical profiles of the fatty acids were markedly varied among individuals. Interestingly, some of fatty acids are detectable only in cats species specifically, but not in other mammals such as mice, dogs, and humans. Chemical profiles of the fatty acids were conserved between fresh and aging urine in each cat. Habituation-dishabituation tests showed that cats can distinguish different compositions of the fatty acids. These results strongly suggest that the fatty acids are key compounds for individual recognition in cats. Our findings will improve our understanding of scent communication in mammals, especially the chemical basis of individual recognition in small felids.

Y7- Investigating the Mechanisms of Singularity of Odorant Receptor Gene Choice

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Odors are detected in the environment through the binding of odorant receptors (ORs), which are expressed by millions of olfactory sensory neurons (OSNs) embedded in the main olfactory epithelium (MOE) that line the nasal cavity. In the mouse, an OSN expresses only one out of 2000 possible OR alleles. The mechanisms behind OR gene choice still remain poorly understood. We will attempt to test whether it is possible for an OSN to express more than one OR and analyze the expression patterns in order to determine if mechanisms for OR choice can only accept one OR locus at a time for expression.

We have shown that the multimerization of a short sequence known to be essential in OR gene choice significantly increases the number of OSNs expressing a defined cloned OR coding sequence (CDS) along with a fluorescent protein from transgenic minigenes. Increasing the probability

of choice for a particular OR may reveal whether more than one choice event is possible in a single OSN and whether we can also uncover OR co-expression.

Therefore, we have developed transgenic mice using our enhancer to drive expression of different OR CDS, and crossed them together to look at how several enhancers may affect OR expression when there is higher competition for choice. Through confocal imaging and immunofluorescence, we can visually count and compare labeled OSNs.

We compared the number of labeled OSNs in offspring of crosses between specific OR-expressing transgenics and gene-targeted strains in which a defined or endogenous OR locus is tagged with a fluorophore. An enhancer-M71 transgene has 25 times more total number of labeled OSNs than endogenous OR MOR23. In comparison, an enhancer-MOR23 transgene has 250 times more total number of labeled OSNs than endogenous OR MOR23. When expressing both enhancer lines for MOR23 and M71 in the same animal, the massive increase in OSN population expressing MOR23 seems to cannibalize probability of choice for transgenic M71 expression and endogenous MOR23, suggesting that the mechanism for choice may be limited in the number of OR loci it can accept for expression.

Some OR co-expression between transgene derived OR labeled cells is also observed, implying that the presence of the enhancer may break the one OR per OSN rule in certain occasions and alter OSN identity. Formation of a glomerulus composed of axons co-expressing two different ORs was also observed, suggesting stable OR co-expression is possible and provides a unique opportunity to characterize this novel identity. This study provides critical insight on the singularity of gene choice and OR expression.

Y8- Ligand binding modes from low-resolution GPCR models and mutagenesis: chicken bitter taste receptor as a test-case

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The bitter taste is one of the basic taste modalities, warning against consuming potential poisons. Bitter compounds activate members of the bitter taste receptor (Tas2r) subfamily of G protein-coupled receptors (GPCRs).[1] The number of functional Tas2rs is species-dependent, with variable numbers of subtypes in different vertebrates.[2] Chicken represent an intriguing minimalistic model because they detect the bitter taste of structurally different molecules with merely three bitter taste receptor subtypes.[3]

Here, I aim to present our recent work focused on the investigation of the binding modes of several known agonists in a representative chicken bitter taste receptor, ggTas2r1.[4] Because of low sequence similarity between ggTas2r1 and crystallized GPCRs (~30% similarity, ~10% identity at most), the combination of computational approaches with site-directed mutagenesis was used to characterize the agonist-bound conformation of ggTas2r1 binding site between TMs 3, 5, 6 and 7. We found that the ligand interaction with N93 in TM3 and/or N247 in TM5, combined with hydrophobic contacts, is typically involved in agonist recognition. Next, the ggTas2r1 structural model was successfully used to identify three quinine analogs (epiquinidine, ethylhydrocupreine, quinidine) as new ggTas2r1 agonists.

Our results demonstrate that the combination of in-silico and in-vitro techniques allows for the rationalization of ligand receptor interactions and the prediction of novel agonists based on the established homology model. This may enable virtual screening of food related activators of chicken bitter taste receptors with implications for the discovery of potentially relevant substances in ecological and agricultural settings. Moreover, the current case study provides a generalizable docking strategy for other GPCRs, where the sequence identity between models and templates is very low.

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POSTER PRESENTATIONS

P1- MITF controls olfactory response through regulation of activity dependent mechanisms

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Microphthalmia associated transcription factor (MITF) is a basic helix-loop-helix-leucine zipper transcription factor essential for the development of melanocytes and mast cells. Mitf is also expressed in the glutamatergic projection

neurons of the mouse olfactory bulb, but its post-mitotic role in these neurons is unknown. Mitf has been shown in melanocytes to be regulated by glutamate signaling. As the nervous system itself is also shaped and regulated by glutamate signaling and the appropriate response to neuronal activity is required for proper functioning of a healthy neuron, a key point of our study was to determine whether MITF takes part in activity-induced responses at the transcriptional level. Using the $Mitf^{mi\text{-}vga9}$ mouse model, we have determined that lack of Mitf leads to an increase in the ability of the $Mitf^{mi\text{-}vga9}$ mouse to distinguish between odors, while its capacity to detect odor is similar to the C57Bl/6J wild type. We have identified tentative target genes of MITF, including several potassium channels sub-units, and show that both Mitf and potassium channel subunits are activity-dependent. Interestingly, we have observed a decrease in Type A- potassium current in $Mitf^{mi\text{-}vga9}$ mouse M/T neurons, and a concomitant increase in neuronal activity of the M/T neurons of the $Mitf^{mi\text{-}vga9}$ mouse. We propose a model, where MITF regulates activity in the olfactory bulb through the regulation of potassium channel sub-units in the M/T neurons. The role of MITF in neuronal homeostasis following activity suggests that MITF plays a major role in long-term selective olfactory habituation.

P2- Brain networks activated during sweet taste processing in children: an fMRI study

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Functional neuroimaging (fMRI) provides a general survey of brain networks involved during sensory and cognitive tasks, and was used here to search possible differences in sweet taste processing in sweet-liker and sweet-disliker 8-11y children. The fMRI data were processed with Independent Component Analysis (ICA) in order to reveal the brain networks and their interactions during sweet solution tasting. 14 children participated in an fMRI paradigm during which they had to taste two solutions of sucrose (0.09M and 0.7M) and a control solution (artificial saliva). Due to children movements and swallowing, we first applied a movement artefact rejection followed by a standard/common pre-processing routine for fMRI data. The ICA enabled us to extract 120 components from which only the ones with bold signal were kept (artifact components were removed). Then, they were sorted considering their anatomical location (auditory, visual, taste, motor, somesthesic cortex, etc.). Temporal correlations were calculated between the taste components and each kind of stimulus and between the components themselves. 29 components with bold signal were obtained and interpreted as cerebral networks. Amongst them, three contained both insula and cingulate cortex, one the striatum and the amygdala and one the orbitofrontal cortex (OFC). One network with the anterior insula and the middle cingulate cortex was correlated with the sweet stimuli. Finally, two groups of temporally close networks were distinguished: the first one with the anterior and posterior insula, middle cingulate cortex, striatum and amygdala and the second group with the anterior insula, the anterior cingulate cortex and the OFC. Within each group, the networks may be involved in a similar cognitive and/or sensory processing. Two main results can be pointed out in our study. First, ICA makes it possible to adequately distinguish the brain networks activated during a taste fMRI paradigm in children. Second, our experiment shows that specific connections may appear between the insular and cingulate cortex, as well as between the amygdala and the striatum. The insula and the cingulate cortex were split into three components revealing three different cerebral processing involvements. Finally, inter-individual comparisons indicate that this approach in the treatment of fMRI data can be useful to better understand differences between sweet likers and sweet dislikers, or between normal- and overweight/obese children.

P3- Systemic Brain Activity May Represent Processes of Odor Quality

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How we discriminate odors is an important question. According to previous studies, perceiving distinct odors begins with activating distinct combination of olfactory receptor (OR) repertoire in olfactory receptor neurons (ORN). This effect consecutively activates distinct ORN and these distinct ORN activities encode in olfactory bulb. Encoded odor information finally is processed in the brain so we can perceive and discriminate odors. However, these studies still remain the question why distinct OR repertoires induce distinct brain activities, and similar neural encoding of olfactory bulb induces distinct odor responses. To solve these questions, it is needed to study in the brain that lastly processes odor information. Thus, this study focused on how similar perceiving odors process in the brain.

We used two odors 2-acetylpyrazine (AP) and 2, 3, 5-trimethly pyrazine (TP) that describe as similar perceptual descriptor and used heptanal (HA) which different category odor compare to AP and TP. To measure the direct brain signal, electroencephalography (EEG) signal was used to understand how participant process odor in the brain. Power spectrum and event related spectral perturbation (ERSP) of theta, alpha, beta and gamma frequency bands are used to verify similarity between AP and TP.

Interestingly, AP and TP induce similar pattern of power spectrum in total electrodes but not in HA. These results

suggest that these two odors activated similar region of brain. Also ERSP results of AP and TP suggest that AP and TP induce similar theta wave pattern in 0~500ms and similar alpha, beta pattern in 500~2000ms. In case of HA, signal became similar with AP and TP at 500~2000ms but not in 0~500ms. These results emphasize that AP and TP may induce similar brain activity especially in 0~500ms but this differences are diminished after 500ms.

Based on these results, similar perceiving odors may induce similar systemic brain activity. Moreover, processing odor quality may end within 500ms.

Key word: olfaction, odor quality, brain, EEG, temporal pattern

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P4- The non-caloric sweeteners cyclamate and saccharin exhibit mutual suppression of bitter taste receptors responsible for their bitter off-taste

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The societies of industrial countries face a growing problem arising from the unrestricted availability of food rich in energy, which results in an increasing number of overweight individuals and the associated metabolic diseases. In order to reduce the energy-intake, a large variety of calorie-reduced products are offered including non-caloric sweeteners that are able to replace natural sugars but maintain the attractive sweet taste. Among the earliest non-caloric sweeteners that have been discovered were saccharin and cyclamate, which unfortunately also share some of the undesired features of many high-potency sweeteners such as limited maximal sweetness and an off-taste at higher concentrations. In order to improve the taste characteristics exerted by the single substances, blends of the two sweeteners were used already in the 1950s in industrial products. However, the mechanism by which these blends resulted in superior taste characteristics remained unknown.

By functional calcium imaging assays we confirmed that saccharin acts as a quite potent agonist of the human bitter taste receptors TAS2R31 and TAS2R43. The co-application of cyclamate resulted in a strong reduction of the saccharin-induced receptor responses demonstrating that cyclamate acts as an antagonist on the two saccharin receptors. Intriguingly, also saccharin was able to suppress the TAS2R1-mediated bitter responses of cyclamate and hence, represents a bitter receptor antagonist as well. Using functional expression of the sweet taste receptor heteromer with saccharin, cyclamate, or blends of the two compounds, we have not observed supra-additive activation of the sweet taste receptor, indicating that the observed elevated maximal sweetness experienced by human probands may not be a TAS1R2/TAS1R3-dependent effect.

P5- Expression and biophysical characterization of the human umami taste receptor

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Umami taste perception is mediated by a heterodimeric receptor composed of the two distinct subunits, T1R1 and T1R3. T1R1 and T1R3 subunits are members of the small family of class C GPCRs. class C GPCRs share a large N-terminal domain (NTD) linked to a transmembrane domain by a cysteine-rich region. The human T1R1/T1R3 receptor responds specifically to L-Glutamate (L-Glu) and L-Aspartate (L-Asp) with a response potentiated by 5' ribonucleotides as IMP or GMP, which also elicit umami taste by themselves. It has been shown that whereas L-Glu binds to the hinge region of the T1R1-NTD and induces its closure, 5' ribonucleotides bind to an adjacent site, near the opening, and stabilises the T1R1-NTD in closed conformation. In contrast to the T1R1-NTD subunit, the functional role of the T1R3-NTD subunit in the umami compound detection remains largely unknown. Here we report the ligand binding properties of the recombinantly expressed T1R1- and T1R3-NTDs. The proteins overexpressed in Escherichia coli as insoluble aggregated protein (inclusion bodies) were solubilised, in vitro refolded and purified by two-step chromatography procedure. Circular dichroism and SEC-MALS analyses demonstrated that purified proteins are correctly refolded as monomeric form. Fluorescence spectroscopy demonstrated that T1R1- and T1R3-NTDs are both able to bind L-Glu and IMP with micromolar affinity. In summary, our results demonstrate the contribution of each subunit to the heterodimeric receptor function for the detection of these umami taste compounds.

P6- The effect of olfactory stimulation on affective valence of dreams and affective state upon waking: preliminary results of a pilot study

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The focus of the presented project are effects of olfactory stimulation during sleep on affective valence of dreams and affective state upon waking. In the pilot study to this project, effects of two "pure olfactory" stimuli, vanillin and thioglycolic acid, which are generally perceived as pleasant and unpleasant, respectively, are investigated. In weekly intervals, participants spend 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.) is recorded and participants are woken up five minutes into the REM sleep phase that occurs around 4 a.m. Immediately

after waking, they are 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 complete the same measures once again upon waking in the morning. Preliminary results in 31 participants show that those receiving olfactory stimulation on the second night reported greater dream pleasantness on that night and morning, regardless of odorant used, compared to their first (adaptation) and third (control) night. However, this was not the case with participants exposed to odor on the third night. Further, compared to the other three conditions, participants exposed to thioglycolic acid (but not vanillin) on the third night reported they felt more awake, peppy, and active upon nocturnal waking and more serene, calm, and relaxed in the morning. All of these effects were independent of whether the participant was aware of the presence of an olfactory stimulus and his or her perceptual ratings of that odor.

P7- Characterisation of the Ligand Binding Pockets of Odorant Binding Proteins for Biosensor Development

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Odorant binding proteins (OBPs) are attractive as recognition elements for creating new types of biosensors (1–7). Selection of suitable proteins that where the binding site can be modified to create proteins with selectivity to desired ligands requires understanding of the binding pocket amino acid residues that are important for ligand-protein interaction. Molecular Modelling tools (including in silico mutagenesis and docking screening techniques) were used to characteristic the binding pockets of AgamOBP1 and AgamOBP47 from malaria mosquito Anopheles gambiae – by using x-ray crystallography structures of the two OBPs (8). The screening identified possibly stable amino acid substitutions within the binding pocket (28 for AgamOBP1) and (79 for AgamOBP47) and docking was then carried out against selected ligands, where 17 of the AgamOBP1 mutants were found to be potentially better proteins compared to WT, but for AgamOBP47 none of its 79 stable mutants was found to be better compared to WT protein. It was found that the binding pockets of these proteins are highly hydrophobic and generally not conducive for amino acids to be replaced. However for given residues where it was feasible to create a substitution, most of the time the new residues should also be hydrophobic in nature. This generic approach allows selection of suitable OBPs for biosensor applications for the detection of different molecules of interest.

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P8- Odour receptor 37 ligands modulates activation of the paraventricular nucleus of the hypothalamus in anxiety-like contexts but with no effect on behaviour.

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The OR37 subfamily of odorant receptors (ORs) represents a rather ancient gene cluster which exists exclusively in mammals. In mouse OR37 genes are clustered into two distinct loci and in contrast to ORs in general, their coding sequences particularly in cluster I are highly conserved within and across species and unusually seem to be under negative selection pressure. Furthermore, mouse OR37 receptor subtypes A, B and C found in cluster I do not send their axons to the typical olfactory cortical areas but project to other brain regions including the paraventricular nucleus of the hypothalamus (PVN) that regulates the hormonal stress response. Recently, mouse OR37 receptors A, B and C have been shown to be activated by the long-chain aliphatic aldehydes pentadecanal, hexadecanal and heptadecanal respectively and reduced stress-induced activation of the PVN suggesting a role in mediating a phenomenon called social buffering.

Towards understanding the biological relevance of mOR37 ligands we initially assessed odour behaviour. Odor habituation/dishabituation test showed no ability to detect and differentiate different OR37 ligands suggesting they lack salience in this context and in unconditioned place preference test mice displayed neutral responses to the OR37 ligands. Behaviour was also analysed in unconditioned anxiety-like behavioural tasks during exposure to OR37 ligands such as open field and zero maze. Interestingly, despite neuroendocrine changes being observed exposure to OR37 ligands does not exert anxiolytic-like behavioural effects. Whilst social buffering is known to modulate physiological and behavioural responses mice do not seem to be aware of OR37 ligands nor do they influence behaviour in an anxiety induced context.

P9- Evolution of olfaction: What did the modern human lose?

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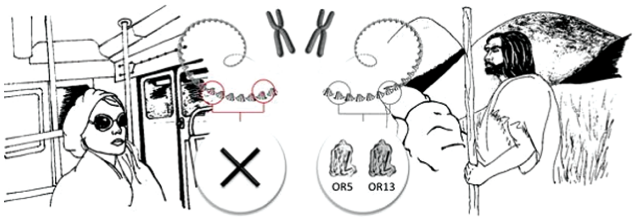
Modern humans perceive odors through the use of odorant receptor (OR) repertoire which contains around 800 different genes. Nearly half of these genes have undergone the pressure of evolution and became pseudogenes, resulting in ~400 functional genes. The OR pseudogenes repertoire is rarely investigated by the olfaction community. Nevertheless, it is the heritage from our ancestors from between 40.000 – 1M years ago. This presents a unique opportunity to better understand various thematic such as their evolution of nutritional or social behavior of hominid.



During this study, we identified two olfactory receptors, OR5 and OR13, which became pseudogenes during the hominid evolutions. We traced the evolutionary tree of these ORs to characterize the function of our ancestors in addition to the ape homologs. These two receptors appeared to be functional in our ancestors and then switched to pseudogenes after Homo Sapiens and Neanderthals separated from Denisovans. These ORs are found intact only in ape species such as gorilla or chimpanzee as they were in Denisovan. To assess the role of these ORs we rebuilt their Denisovan version by synthesizing their corresponding nucleotide sequences inserted into a mammalian expression vector. Next, site directed mutagenesis was applied to obtain additional ancestors and ape orthologs. Then, we monitored their in vitro responses to a large set of odorant molecules that we hypothesized to be pertinent regarding the living environment of our ancestors. We located the changes, that occurred during evolution, in a three-dimensional model to infer critical residues for ligand binding and signal transduction. It allowed us to hypothesize how evolution targeted specific sites in OR sequences.

From our knowledge, this is the very first time that ORs, pseudogenized in human lineage after our divergence with Denisovans, have been resuscitated and functionally assessed. This study brings new insights into our understanding of this extinct species.

P10- Investigation of a new chemosensory method using three taste inhibitors in domestic cats: examples of bitter, umami and kokumi taste perception

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Taste inhibitors are commonly used to study taste mechanisms in humans and rodents. We tried this technique to understand the taste perception in the domestic cat. Three tastes were selected: bitter, umami and kokumi, potentially very important to cats, due to their strict carnivorous nature. We used three taste inhibitors: ZnSO4, which inhibits bitter taste; gurmarin, a protein from the plant Gymnema sylvestre, which inhibits umami taste and the NPS-2143, an antagonist of the kokumi CaSR receptor. The method of administering inhibitors to cats was specifically developed for this study, to comply with voluntary feeding, but still ensuring the intake of a specific amount of inhibitor. Specific jellies were proposed to a panel of 40 cats and followed by a versus test opposing cat dry diets coated with specially formulated palatability factors, representative of the three basic tastes to be studied. We used quinine for bitterness, IMP-GMP / sodium mono-glutamate for umami, and the same association enriched with glutathione for kokumi. At the panel level, the umami and kokumi foods were very significantly more consumed than the controls but consumption of gurmarin or NPS-2143 before the meal did not reduce this preference. The bitter food containing quinine was clearly rejected by cats, with no effect of ZnSO4. These initial results indicate that the three inhibitors tested in this study have no strong effect on cats. It would be interesting to compare the gene sequence of the taste receptors in humans, rats, mice and cats to check if the genetic variations across species may explain these first results. Moreover, the analysis of the first individual data suggests that like in other species, there may be different sensitivities between individuals, leading to different perception of taste and therefore to different efficiencies of the inhibitors

P11- Cisplatin chemotherapy decreases hedonic perception of sweet taste in bronchial cancer patients

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Chemotherapy side effects include physiological, psychological and social disturbances in cancer patients. Sensory impairments have also been mentioned, including those affecting the olfactory and gustatory systems. In a previous study (Joussain et al., 2013), we showed a decrease in pleasantness for food odors in patients with chemotherapy treatment. The aim of the present study was to to examine whether these hedonic changes can be extended to another prominent chemosensory system, namely gustation. We further asked whether changes in gustatory perception in cancer patients can be observed at the threshold level. To ask this, we used non-analyzed data from the study of Joussain et al. whereby 15 bronchial cancer patients receiving cisplatin and 15 control participants were tested gustatory perception (using taste strips test, assessing detection, intensity, identification and pleasantness of 2 basic gustatory stimuli, saltiness and sweetness). Patients were tested under two different sessions: the first session of tests was conducted before the beginning of the treatment, and the second one 6 weeks later, after the third cycle of chemotherapy. Controls were tested under the same protocol, with two sessions separated by 6 weeks. Results showed that pleasantness of sweetness (after vs. before chemotherapy) was significantly lower in patients than in controls ($p=0.01$). No such difference was observed for the salty stimulus, or for the remaining tasks (detection, intensity and identification, $p>0.05$ in all cases). Interestingly, odor threshold was not affected by chemotherapy ($p>0.05$). Taken together, these findings suggest that the sensory - and in particular the chemosensory - changes that accompany cisplatin chemotherapy are more likely to be a consequence of a change in the functioning of the networks involved in emotional processing of olfactory and gustatory information rather than a change at the peripheral levels. Future studies conducted on larger group of patients will enable understanding how these hedonic olfactory and gustatory modifications influence food intake and habits of cancer patients.

P12- A model of stochastic computation in neurons describes and quantifies the emergent psychophysical phenomena of taste, odour and flavor

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Human psychophysical taste intensity data can be described by sigmoid continuous curves from several families of probability functions. One of these probability functions suggests spatial summation in neurons. Computer simulation of arrays of taste receptor cells, with merging and spatial summation of spike-trains, exposes mechanisms for perceived magnitude of taste intensity, recognition thresholds and difference thresholds, which fit published data. A further spatial summation of the five taste modalities and aroma exposes mechanisms for perceived flavour intensity, mutual suppression in taste, detection thresholds and odour-induced taste enhancement.

An extension of these methods, to simulation of merging and lateral inhibition in the olfactory glomeruli, suggests a quasi-logarithmic mechanism for perceived odour intensity, odour detection thresholds and difference thresholds. The model implies that individual difference in taste and odour perception is mostly encoded by plastic rearrangement in the spatial summation steps, perhaps as a means of optimising intensity measurements to the current dietary environment.

Optimisation through a computed passive gain ratio mimics published measures of sensory liking/pleasantness, as a perception of an optimum intensity. Is this a fundamental mechanism of 'mere exposure' in taste and odour?

This treatment of gustatory and olfactory processing, as a stochastic neural computing system, forms a framework to resolve previously disparate observations into a cohesive system of related, predictable phenomena, by which individual perception, and distributions within and between populations can be characterised and understood. Industrial and public health consequences of the model will be presented.

P13- Minute impurities contribute significantly to olfactory receptor ligand studies: tales from testing the vibration theory

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Several studies have attempted to test the vibrational hypothesis of odorant receptor activation in behavioral and physiological studies using deuterated compounds as odorants. The results have been mixed. Here we attempted to test how deuterated compounds activate odorant receptors using calcium imaging of the fruit fly antennal lobe. We found specific activation of one area of the AL corresponding to inputs from a specific receptor. However, upon more detailed analysis, we discovered that an impurity of 0.0006% ethyl acetate in a chemical sample of benzaldehyde-d_5 was entirely responsible for a sizable odorant-evoked response in Drosophila melanogaster olfactory receptor cells expressing dOr42b. Without gas chromatographic purification within the experimental setup, this impurity would have created a difference in the responses of deuterated and non-deuterated benzaldehyde, suggesting that dOr42b be a vibration sensitive receptor, which we show here not to be the case. Our results point to a broad problem in the literature on use of

non GC-pure compounds to test receptor selectivity, and we suggest how the limitations can be overcome in future studies.

P14- The (Mis)Measurement of Sniffing in Olfaction-fMRI

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Measurement of nasal airflow during olfactory fMRI scans is essential because the act of sniffing alone, in the absence of odor, induces vast activation in olfactory regions. Moreover, whereas intense and unpleasant odorants are automatically sampled with moderate sniffs, mild and pleasant odorants are automatically sampled with vigorous sniffs. Thus, in order to dissociate sniff-induced from odor-induced patterns of activity, it is important to assure that sniffing is constant across different odor conditions. A common method for evaluating odorant-dependent sniffing in olfaction-fMRI studies is using respiratory belts placed around the abdomen or chest. Such belts, however, are notoriously insensitive to the small perturbations in nasal airflow associated with changes in odor content, changes that are best measured with direct assessments of nasal flow [Johnson BN, 2006]. Thus, we test the hypothesis that using respiratory belts to measure nasal airflow in fMRI is a potential source for error in interpretation of odor-induced brain activation patterns.

Nine subjects were each scanned (Siemens Tim-Trio 3T, 58 2.3mm slices, TR=2000 ms, TE=25 ms) in four runs where respiration was monitored simultaneously; directly with spirometer (ADInstruments) and indirectly with respiratory belts around the chest and abdomen (AcqKnowledge, BIOPAC Systems). We used an olfactometer to generate three pleasant odorants: rose, banana, and orange, and two unpleasant odorants: asafoetida and bacon. At each trial, subjects sniffed for the duration of a 2-second auditory cue (note that the addition of this consistent external cue biases the behavior against our hypothesis). In each run, 15 odorants were delivered in random order, with a random interstimulus interval (ISI) of 26–30 seconds. Subjects rated odor pleasantness after each trial. Breathing during the ISI was oral (velopharyngeal closure) in runs #1 and #3, and nasal in runs #2 and #4.

Belt data was unavailable for 8 of 36 runs. Our initial results indicate that although subjects were trained to take the same-sized sniff at each trial, the direct spirometer-based measure of nasal respiration revealed significantly reduced sniff volume for unpleasant versus pleasant odors, both with oral ISI breathing (Norm. Vol. Pl. = 1.36 ± 0.42, Norm. Vol. UPl. = 1.25 ± 0.38, $t(8) = 1.92$, $p < 0.05$) and nasal ISI breathing (Norm. Vol. Pl. = 1.27 ± 0.15, Norm. Vol. UPl. = 1.17 ± 0.18, $t(8) = 1.87$, $p < 0.05$). In turn, respiratory belts revealed the same effect for oral ISI breathing (Norm. Vol. Pl. = 0.55 ± 0.16, Norm. Vol. UPl. = 0.51 ± 0.18,$t(7) = 2.51$, $p < 0.02$) but not for nasal ISI breathing (Norm. Vol. Pl. = 0.47 ± 0.18, Norm. Vol. UPl. = 0.48 ± 0.16,$t(6) = -0.43$, $p = 0.66$). More critically, correcting for respiratory volume based on either direct or indirect measures of respiration (spirometer vs. belts) gave rise to significantly altered patterns of "odor induced" brain activity. These initial pilot results highlight the significance of accurately measuring nasal airflow in fMRI.

P15- Discrimination of dog palatant solutions using human olfactory receptors platform

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Food choice is guided by various factors as senses, physiological or emotional states. A strong relationship has been observed between food choice and the sense of olfaction in dogs. In order to better understand the pleasant compounds perceived and recognized as such by the dog, it is important to have an analysis method of palatant solutions, precise and accurate to the products. Some aroma analysis methodologies allow to detect most of the volatile compounds present in the food products, but they don't allow to determine the molecules really perceived by the dog and their impact on food preferences. New methodologies are necessary to achieve this goal, based on the olfaction physiology and mechanisms, in order to be closer to the perception. An innovative study was conducted by Diana Pet Food and ChemCom to evaluate the interest of using olfactory receptors, as sensors, in the characterization and discrimination of dog palatant solutions. Five different dog palatant solutions (A, B, C, D and E) were assessed, according to diverse protocols. First, palatability of these solutions, coated on dry dog kibbles, was evaluated by food preference tests, performed on dog panels (60 dogs). Second, discrimination tests on these five solutions were performed on human panel of 30 assessors. These solutions were finally analyzed on olfactory receptors platform composed of 135 deorphanized human olfactory receptors. The five solutions were discriminated by the dog and the human panels. Moreover, they were perceived with different levels of palatability by the dogs, i.e, products A, C and E were preferred to B and D. On the olfactory receptors platform, a specific profile of activation was established for each palatant solution, leading to the product discrimination. In term of discrimination, results obtained with the human olfactory receptors platform were comparable to these obtained by dog and human panels. The profile of human olfactory receptor activation of each solution was based on the nature and the activation level of each receptor, giving thus complementary information on the palatant solutions, eventually on the perceived volatile compounds present specifically in a solution, and probably their impact on the palatability of the product. Based on these data, it would appear that the use of olfactory receptors as sensors

could highlight some molecules potentially implicated in the palatability of food products. This would be relevant for a better characterization of products, and the development of new petfood products.

P16- Impaired olfactory brain response to odors in patients with traumatic brain injury

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Background: Traumatic brain injury (TBI) as a major public health problem may lead to olfactory dysfunction. However, less is known about brain responses to odors in TBI olfactory loss patients.
Methods: Seventeen healthy controls and forty TBI olfactory dysfunctional patients (19 hyposmic and 21 functional anosmic patients) were enrolled in the study. Olfactory performances were measured using the "Sniffn' Sticks" test. Olfactory brain activations in response to odors were measured using functional magnetic resonance imaging.
Results: Compared to healthy controls, the TBI patients had reduced olfactory functions (tested with the Sniffin' Sticks battery), as well as decreased odor-induced brain activations in primary and secondary olfactory regions. TBI patients with functional anosmia showed further reduced activation in the orbito-frontal cortex and putamen compared with hyposmic patients. In addition, there was a negative correlation between the time since TBI and brain responses to odors in the insula cortex ($r = -0.58$, $p < 0.001$).
Conclusion: Results from the current study are evidence for the impairment of functional brain activation to odor perception among TBI patients, and the duration of TBI had a further impact on the severity of the olfactory dysfunction. This pattern of damage seems to be more severe than what has been reported in patients with postinfectious olfactory loss.
Key Words: Traumatic brain injury; Olfaction dysfunction; Odor perception; fMRI

P17- Somatosensory Response to Trigeminal Stimulation: A Functional Near-Infrared Spectroscopy Study

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Smelling entails not only olfactory but also less investigated trigeminal processes. Virtually all airborne chemical substances stimulate the trigeminal nerve when being delivered in high concentrations. This stimulation can elicit perceptions in the upper respiratory tract e.g. burning, stinging or coolness.

In humans, previous functional magnet resonance imaging (fMRI) studies have revealed a cortical network called 'the pain matrix' that is being activated by trigeminal stimulation. Within this network the primary and secondary somatosensory cortices (SI and SII) play important roles. Even though fMRI offers high spatial resolutions, studying chemoreception using fMRI is costly and methodologically challenging due to the restrictive scanner environment, not allowing any magnetic metals near the participant, and strong motion-related artifacts.

Therefore, it is of great interest to explore alternative imaging techniques, their capabilities and limits of measuring chemosensory-related brain activity. Functional near-infrared spectroscopy (fNIRS) is an optical imaging technique suitable for measuring relative hemodynamic changes in superficial cortical brain structures. fNIRS combines acceptable spatial resolution (~3 cm) with higher temporal resolution (~10 Hz) and motion tolerance than fMRI. Thus, it might be possible to target areas related to trigeminal perception.

The current study aims at using fNIRS to measure trigeminally evoked neuronal activity over the SI and SII using the well-characterized trigeminal irritant acetic acid which perceptually elicits a stinging sensation. Eleven healthy subjects (age: M=26.4; female: 5) were exposed to acetic acid in an event-related design using a respiration-synchronized olfactometer.

First results indicate that stimulation with acetic acid can lead to significant evoked changes in oxygenated and deoxygenated hemoglobin in SI and SII when contrasted against pure air stimulation ($t(10)=3.29$, $p = 0.008$).

This is first evidence that fNIRS might be a suitable imaging technique to assess chemosensory neuronal correlates in an objective, nonverbal, easy, and comparably inexpensive manner.

P18- Human Olfactory Receptors: A journey from cell engineering for efficient in vitro functional assays to effective antagonists in human sensory assay

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Many odorant compounds are perceived as unpleasant. They can be present in different contexts such as body-, home-, factory-, material-, or fabric-emitted odors, so that humans are daily exposed to this olfactory pollution. In addition, odorant compounds can also taint food or beverages. Malodor and off-note counteraction is a daily challenge for many different industries.

The first step of the odor perception corresponds to the interaction of olfactory receptors (ORs) with odorant molecules. Therefore, a selective inhibition of the ORs by weakly odorant or odorless antagonists represents an innovative solution to malodor issues. The identification of such odor blockers requires an efficient technological platform to first fish out the receptors that interact with a malodor of interest and second, to screen libraries of potential antagonists of these ORs.

Here, we describe a concrete example of such a discovery process. A proprietary cells line, expressing RTP1A1 and RTP2, optimized for functional expression of ORs has been set up at ChemCom and used to screen large compound libraries (> 7,000) to identify agonists for human ORs. At this stage, more than 130 out of 396 human ORs and 3 out of 6 human TAARs have been deorphanized (i.e. activated by at least one odorant compound) when using a Luciferase-based gene reporter assay. The functional responses obtained with our cell line was shown to outperform those obtained with alternative models (HANA3A and HEK293T parental line).

The same OR functional expression system has also been used to identify antagonists for the well characterized receptor OR7D4. This human OR was shown to mediate the perception of androstenone, an odorant steroid found in male sweat and urine. Screening of low odor compound libraries led to the identification of different antagonists. One, CC-04893, presenting a particularly faint odor, was further characterized in vitro on OR7D4 using androstenone or its structural analog androstadienone as activator. Our results indicate that CC-04893 is a potent antagonist and blocks the response elicited by both activators. Finally, when assessed by panelists sensitive to androstenone odor, the antagonist was found to strongly reduced the perception of the characteristic sweaty, urine-like note of this steroid.

Our results demonstrate the efficiency and usefulness of the olfactory receptor-based in vitro approach to identify and develop new modulators of odor perception, and its application to key malodor suppression.

P19- Bitter taste receptor agonists mediate contraction and relaxation in murine gallbladder smooth muscle

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Taste receptors have extra oral functions. Previously we characterized numerous chemosensory cholinergic cells (brush cells) in the gallbladder epithelium, supposed to sense chemical irritants and bacteria through bitter taste receptors (TAS2Rs). Moreover, TAS2Rs are also expressed on smooth muscle cells (SMC) of various organs where they are not linked to the canonical taste transduction cascade. We here assessed the contractile response of the mouse gallbladder to bitter agonists, which might be triggered directly or indirectly via brush cells and connecting nerve fibers.

Expression of TAS2Rs in the murine gallbladder was assessed by RT-PCR. Changes in intracellular calcium concentration in response to different bitter agonists was measured fluorometrically in isolated gallbladder SMC by confocal laser scanning microscopy. Contractile responses to TAS2R agonists were recorded in trpm5$^{-/-}$ (cation channel being essential for canonical bitter taste transduction), Tas2r143/135/126$^{-/-}$ and their corresponding wild type mice strains using organ-baths.

Tas2R108, Tas2R126, Tas2R135, Tas2R137, Tas2R138 and Tas2R143 were expressed in the murine gallbladder. Increased calcium responses were observed for the bitter agonists denatonium, quinine and dextromethorphan, the largest increase seen for dextromethorphan. The bitter compounds denatonium, noscapine and quinine induced relaxation of precontracted (0.1 μM CCK) gallbladders in the organ-bath, whereas dextromethorphan causes contraction in a dose-dependent manner at 1–100 μM and relaxation at 1–5 mM. All these responses were unaffected in gallbladders from trpm5$^{-/-}$ mice. Both dextromethorphan contraction and relaxation were not sensitive to the cholinergic muscarinic blocker atropine (1 μM), U73122 (10 μM) as specific PLCβ2 inhibitor, muscarinic and purinergic blockers (suramin 300 μM + PPADS 100 μM + atropine 1 μM), or blockade of neural action potential generation (TTX 1 μM). Contraction and relaxation to dextromethorphan were, however, markedly impaired by the selective L-type Ca^{2+} channel inhibitor nifedipine (10 μM) or the selective tetrodotoxin-resistant Nav1.8 subtype blocker, A-803467 (5 μM). Relaxation to dextromethorphan (10 μM) and denatonium (10 μM), but not that mediated by quinine, was partially affected in Tas2r143/135/126$^{-/-}$ mice.

TAS2R agonists have profound effects on gallbladder SMC mainly through a pathway that is independent of cholinergic brush cells and partially dependent on nerve fibers and utilizes a signaling cascade that differs from that in oropharyngeal taste cells.

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P20- The study of odor adaptation in human brain revealed by olfactory event-related potential

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Odor adaptation is the decreased intensity perception of an odor when the odor is continuously offered. Odor adaptation has important roles in our daily life, such as desensitization for unpleasant odors or odor discrimination from background odors. Even though the importance of the odor adaptation, our understanding of odor adaptation in central nervous system remain unclear. According to previous studies, odor adaptation is related to the decreased activation of olfactory receptor neurons (ORNs) in the olfactory

epithelium (OE). However, there are less evidences how this reduced signal process in the brain. Therefore, we focus on how odor adaptation processes in central nervous system.

To understand the central processing of odor adaptation, we performed EEG experiments to analyze event-related potential (ERP) N1 component. N1 is one of the widely studied components of the ERP studies. N1 is known as represent processing of exogenous stimulus in dependence on the endogenous state and altered when stimulation intensity is changed. In odor stimulation, N1 is changed depending on odor concentration. For these reasons, we focused on how N1 is related to odor adaptation. We used geraniol for odor stimulation. We set three different pre-stimulation conditions: geraniol, 2-acetylpyrazine and distill water. Geraniol is odor adaptation condition and other two are control conditions. We set 15 sec for pre-stimulations, 2 sec for odor stimulation, then 30 sec for rest period.

Interestingly, in the adaptation condition, the decreased amplitude of the N1 is observed than the amplitude in the control conditions. These results related with behavior results that the intensity perception of geraniol is decreased than the perception of other two conditions.

These findings suggest that odor adaptation may also reduce responses of olfactory processing in the brain and these processes may be represented by the change of N1 component.

Keyword: Odor adaptation, EEG, event-related potential, N1 component

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P21- Axillary Extracts Affect the Length of Menstrual Cycle in Reproductive Age Women and Pre-Menopausal Women

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Human male underarm secretions, when applied to women recipients, alter the length and timing of the menstrual cycle. These effects are thought to arise from exposure to primer pheromones that are produced in the underarm (Preti et al., 2003). Specific aim of our study was to investigate the influence of male axillary extracts on the length of menstrual cycle of premenopausal women in comparison with women of reproductive age. Total of 43 women, age of 21–51, participated in our study. We monitored the length of the menstrual cycle for each test subject for four months before experiment and followed monitoring for two months after the experiment. We applied axillary extracts/or diluent on the upper lip three times a week for 6 hours in the morning for duration of 9weeks. Women of 21–44 years old were subdivided into three groups: with menstrual cycle length of 26–32 days (1); menstrual cycles shorter than 26 days (2) and with cycles longer than 32 days (3). Experimental data from women over 45 years old were analyzed separately. Male axillary extracts did not affect significantly length and regularity of menstrual cycle in women with normal and regular cycles ($n=12$, $p> 0.1$). At the same time we observed significantly shorter menstrual cycles ($n=12$, $p< 0.01$) under male axillary extracts applications in women with cycles longer that 32 days. For women with menstrual cycle length less than 26 days, we also did not observe statistically significant changes under male axillary extracts treatment ($n=9$). In premenopausal women male axillary extracts applications caused significant shortening of the menstrual cycle ($n=10$, $p< 0.01$). Also we observed a tendency for more regular cycles for this group of test subjects.

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P22- Cross-fostering of Mus musculus and M. spicilegus: Effects on the olfactory sexual preference and neuronal activity to species-specific odors

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We examined the role of early olfactory experience in development of adult odor preferences in two closely related species (Mus musculus wagneri and M. spicilegus). Pups (males) of both species were reciprocally cross-fostered shortly after the birth. At 30 days of age males were weaned and individually housed in cages. At 2 months of age males were tested for response to con- and heterospecific urine odors of estrous and anestrous females using two-choice tests. Males of control (non-fostered) group investigated urine odor of conspecific females significantly longer than urine odor of heterospecific females. Cross-fostered male M. spicilegus preferred investigating the odor of anestrous females of M. m. wagneri compared to conspecific females and did not demonstrate differences in time investigation of con- and heterospecific odors of estrous females. We also examined neuronal activity of cross-fostered and control males using fMRI, namely manganese-enhanced magnetic resonance imaging (MEMRI), to reveal simultaneously the responses in main (MOB) and accessory olfactory bulbs (AOB) to con- and heterospecific female odors. Urine odor of conspecific females, a complicated mixture of pheromones and odorants, elicited significant signals in the dorsal region of the posterior part of the MOB and some region of the AOB. Urine odor of heterospecific females did not elicit signal in the AOB whereas it did elicit signals in the dorsal region in the anterior part of

the MOB. Urine patterns of activation were similar in M. m. wagneri and M. spicilegus males. Early olfactory experience modified the patterns of neuronal activation in response to the heterospecific urine odor of females. In cross-fostered males of M. m. wagneri urine odor of both con- and heterospecific females elicited significant signals in AOB. Results are discussed regarding the current knowledge in the field of the formation of isolating mechanisms in ontogenesis as well as the differences in the chemical composition of urine of two Mus species. The research was supported by the Russian Science Foundation grant №16-14-10269.

P23- Evaluation of various cell-based assays for olfactory receptors

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Our sense of smell plays an important role in our daily lives especially in food consumption, influencing both food choice and the amount of food intake. The last three decades have seen great advances in the field of olfaction, beginning with the identification of olfactory receptors (ORs) and their signaling pathways. However the pairing of individual ORs with their cognate ligands has been progressing slowly. This task has been complicated by difficulties in establishing a general functional assay system for ORs due to low expression levels of receptors in heterologous systems, high background signals of some ORs, and low reproducibility for others. We therefore set out to generate, evaluate, and compare different cell-based assays for ORs.

We first used CRISPR/Cas9 genome editing to generate reporter cell lines expressing RTP1s from its endogenous genomic locus, as co-expression of RTP1s with ORs is necessary for the transport of the receptor proteins to the plasma membrane. Using these cell lines, we then set up four different functional assays for ORs. These included the well-established and frequently used CRE-luciferase assay, in which receptor-induced cAMP generation leads to the expression of a luciferase reporter. We also adapted for ORs two recently developed technologies for the study of Gαs-coupled receptors and the detection of cAMP, GloSensor and protein kinase A (PKA)-NanoBiT. In addition, we used our proprietary chAMPion assay, which measures cAMP levels indirectly via calcium influx through cyclic nucleotide gated channels, thereby replicating the native OR signaling pathway. We then selected ten human ORs with published ligands to be tested in all four assays. General assay performance was good for three of the four assays with only the PKA-NanoBiT assay providing a too low and unstable assay window. Depending on the OR analyzed, basal signals and signal-to-background ratios varied between the assays, as did assay sensitivity. While one assay worked better for one OR, another assay was more suitable for another OR. Also, not all of the ten human ORs tested showed responses in our experiments. In conclusion, while neither assay alone is a general best assay for all ORs, a combination of two assays, CRE-luciferase and chAMPion assays, promises to yield good results for most ORs.

P24- Co-expression of taste receptors and CaSR in mouse taste cells

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The sense of taste provides valuable information about nutritional quality of food. Human and mice can sense five basic tastes, sweet, bitter, sour, salty and umami. Receptors for each of the basic tastes are thought to be expressed in taste bud cells. It is well established that sweet and bitter tastes are elicited by the activation of T1R2+T1R3 and T2Rs, respectively. The major receptor for umami is considered T1R1+T1R3 and it demonstrated that mGluR1 and mGluR4 also contribute to umami taste. Additionally it is suggested that free fatty acid receptors contribute to the "taste of fat". We previously demonstrated that the orally administered agonists/modulators of calcium-sensing receptor (CaSR) launch orosensory mechanisms. For instance, in human sensory studies, γ-glutamyl valyl glycine (γEVG) enhances the intensities of umami, sweet and salty tastes, also modify the continuity, mouth-fullness and "thickness" in a CaSR-dependent manner. These phenomena are called 'kokumi'. Importantly, the kokumi substances do not elicit any taste itself. To elucidate the molecular mechanisms of kokumi, we characterized CaSR-expressing taste cells and detected that various taste receptor molecules are co-expressed with CaSR. Double labeled experiments were performed using in situ hybridization and/or immunohistochemistry in mouse vallate papillae. Collectively, our data show that CaSR was expressed in type II and type III taste cells. Interestingly, CaSR-expressing cells form different subset of T1R3-expressing umami or sweet taste cells. CaSR-expressing cells that do not express T1R3 co-expressed other taste receptors. Our results suggest the possibility that CaSR may contribute to multiple functions of taste cells and that kokumi substances might affect taste perception through coordinated and complex action on individual taste cells.

P25- Vasopressinergic actions in the olfactory bulb: insights in form and function

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The neuropeptide vasopressin (VP) is a major mediator of vertebrate social behavior, best described within limbic brain

areas. However, the intrinsic VP system in the main olfactory bulb (MOB) and therewith its influence on volatile social odor processing are relatively unexplored. So far, bulbar VP cells are described as glutamatergic external tufted cells and VP has been shown to reduce output from mitral cells in-vivo. However, the detailed physiological mechanisms, i.e. how VP modulates the encoding of social odor information on the cellular network level is unclear. Our initial aim was to further characterize morphological and physiological details of the MOB VP system.

Morphological analysis revealed that VP cells bear a distinct primary dendrite taking a tortuous route before entering a single glomerulus and forming a tuft similar to mitral and tufted cells. Their lateral dendrites/axons spread widely and deeply in the external plexiform layer including single axonal fibers reaching the internal plexiform layer extending to far reaching projections in anterior and posterior directions. To further explore potential functions of the VP cell apical tuft, we characterized its branching pattern within its parent glomerulus. This analysis yielded a uniform, widespread innervation, comparable to glomerular dendritic tufts of mitral cells.

Whole cell patch clamp recordings revealed that depolarizing currents applied to VP cells resulted in non-bursting, accommodating firing patterns which do not imply pacemaker activity, but direct excitation from the olfactory nerve (ON). Therefore, we performed whole cell recordings from VP cells and electrically stimulated the ON axons anterior to a VP cell's parent glomerulus. Surprisingly, this stimulation did not result in direct monosynaptic excitation but always induced $GABA_A$ receptor-mediated IPSPs (11 out of 11 cells). Bicuculline blocked these IPSPs and unmasked barrages of depolarizing potentials (n = 10). Modulation of ON-evoked IPSPs in VP cells via application of 1 µM VP could not be observed (n=7).

To investigate the underlying mechanism of Mitral cell output reduction, we have demonstrated that this effect is most likely due to a depression of ON inputs on mitral cells, since application of 1 µM of VP in vitro produces a reversible average decrease of ON-evoked mitral cell EPSPs to 82 ± 5 % of baseline (n = 7, $P < 0.05$).

Due to their proximity, mitral cell glomerular dendritic tufts may be target areas of VP release sites located in their tufts. Thus, we additionally characterized the spread of action potential-induced Ca^{2+} signals within apical dendrites of VP neurons. Contrary to our expectation, we consistently observed very small dendritic Ca^{2+} transients in response to individual back propagating action potentials and a moderate increase in dF/F in response to prolonged, somatically evoked action potential trains ($p < 0.05$, n=15 cells).

Current studies are underway to further profile form and function of this rather unusual peptidergic interneuron subtype in the MOB, including electron microscopic structural analysis and behavioral pharmacology.

P26- A flavor modifier, γ-glutamyl-valyl-glycine, has enhancement effect on the response of trigeminal neuronal cells

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Gustation and oral somesthesia are key factors for oral sensation. The receptors for basic tastes and pungent substances are expressed in specific taste cells or somatosensory trigeminal neurons. We have discovered substances that appear to modify these oral sensations. For instance, in human sensory studies, γ-glutamyl-valyl-glycine (γEVG) enhances the intensity of umami, sweet, and salty tastes, although it has no taste itself. Calcium-sensing receptor (CaSR), a receptor for γEVG, is expressed in a subset of taste cells that presumably are involved this enhancement of taste. Using immunohistochemistry and single-cell RT-PCR, we recently observed CaSR expression in trigeminal ganglia. Most CaSR-expressing small cells co-express TRPV1 (a capsaicin receptor) and TRPA1 (an allyl isothiocyanate receptor). In intracellular Ca^{2+} imaging, the amplitude with allyl isothiocyanate-induced response were enhanced with pretreatment of γEVG in a subset of the neuronal cells. Moreover, a model substance, which has similar physical properties to γEVG, penetrated into keratinous layer of tongue epithelium. These results suggest that γEVG can modify pungent and mechano/thermo sensations. Collectively, the results suggest that γEVG is a multimodal modifier for oral sensation.

P27- A mercaptan emitted from excretions of domestic cats alerts other animals to their presences

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Scent emitted from animal excretions such as urine and feces provides important signals for species, sex, age, and individual recognitions in territorial animals. Their excretions contain several volatile compounds, some of which function as scent signals with information of territorial owners. Volatile compounds whose levels are higher in limited species and male/female may act as scent signals for conspecific and sex recognitions, respectively. However, such compounds have been identified only in laboratory rodents and limited mammalian species. In this study, we examined volatile chemical profiles of domestic cat feces used for territorial marking, and also compared between cat fecal odor and anus odor through which animals obtain species information on scent owners from visual cues before sniffing. GC-MS detected 3-mercapto-3-methylbutanol (MMB), which is a derivative of the unusual amino acid known as felinine, with short-chain free fatty acids (FFAs) in cat feces. MMB emission

rates from feces showed sex differences (male > female) and dynamic temporal changes during aging. Behavioral bioassays showed that male cats can distinguish fecal odors with and without MMB. Anus odor was composed of FFAs whose contents were varied between individuals, but did not contain species-specific compounds like MMB. Cats distinguished different variations of FFAs. These findings will improve our understanding of scent signals emitted from excretions in mammals, especially volatile compounds emitted from their excretions contain specific compounds that may alert other mammals to species, sex, and temporal trace of scent owners, while individual information rather than species information may be enhanced in body odor.

P28- Olfactory impairment and cognitive disabilities in a subject with Parkinson's disease: a case report

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Background: Parkinson's disease (PD) is a neurodegenerative disorder characterized by motor symptoms like bradykinesia, rigidity, tremor and postural instability. PD is usually associated with non motor symptoms (NMS) that include olfactory and taste dysfunction, neuropsychiatric symptoms as apathy, anxiety and cognitive impairment, sleep problems and autonomic dysfunction (1). The olfactory dysfunction is considered the most common NMS in PD and it has been well known since 1975 (2, 3). Interestingly, this impairment often precedes motor symptoms of parkinsonism and is investigated as a potential biomarker in subjects at risk of PD. Aim of the study was to describe a peculiar PD patient with history of olfactory dysfunction, chronic cerebral vascular disease and mild cognitive impairment.

Materials and method: A 46-years-old man affected by mild rest tremor associated to chronic cerebrovascular disease, severe obesity and mild cognitive impairment went to our Center. Motor impairment was assessed by mean of the Modified Hoehn and Yahr (HY) Stage and the Unified PD Rating Scale (UPDRS) part III. Instead, olfactory function was assessed by mean of Sniffin' Sticks Extended Test taking into consideration three different functions: odor threshold (OT), discrimination (OD), identification (OI) and the TDI score (4).

Results: The olfactory function assessment indicated functional anosmia with an impairment in and TDI score. Neurological examination showed mild bilateral bradikynesia, while rigidity was detected in his left hemibody with mild rest tremor. Brain MRI showed chronic cerebral vascular disease, while SPECT DATSCAN exhibited reduced dopaminergic innervations in the right putamen.

Discussion and Conclusion: The present study, while confirms that olfactory function allows an early PD detection, also suggests that olfactory impairment and cognitive disabilities make possible differential diagnosis of neurodegenerative disorders.

References:

1) Chaudhuri et al., 2006. 2) Ansari and Johnson, 1975. 3) Sobel et al., 2001. 4) Hummel et al., 2007.

P29- Chemosensory stress signals reduce trust in women

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Previous studies have shown various physiological effects of chemosensory stress signals on women. However, behavioral effects in social interactions have rarely been addressed. Aim of this study was to examine the effects of chemosensory stress signals on trust behavior in social interactions.

A total of 39 women were asked to participate in a trust game with a fictional co-player, they believed to be real. To assess trust behavior, participants were given the choice to transfer money (between zero and three Euros) to their co-player. In order to create a situation, in which participants believed they could receive a retransfer of money back from their co-players, they were told, their transfer money was tripled and the co-player was free to return any amount of money. Chemosensory stress signals were collected via cotton pads from 22 men, participating in a modified version of the Trier Social Stress Test for groups. In a control condition, the same men participated in a mild exercise to collect sport sweat. As a third condition participants in the trust game were presented with pure, non-odorous cotton. The three odors were presented via a 3-channel-olfactometer for three seconds each (45 ml/s), with participants rating intensity and pleasantness of the three odors. In the following trust game, participants inhaled one of the three odors just prior to making their decision on how much money to transfer to their co-player, repeating the procedure for each odor in each participant.

Results show, that participants transferred less money in the "stress" condition, compared to the "sport" ($p = .001$) and "cotton" conditions ($p = .001$). "Sport" and "cotton" conditions did not differ in the money transferred to the opponent. Stress sweat was rated as less pleasant than sport sweat ($p = .001$) or pure cotton ($p < .001$). Intensity ratings were higher for stress sweat as compared to pure cotton ($p < .001$), and higher for sport sweat as compared to pure cotton ($p = .001$). Intensity ratings for stress and sport sweat did not differ.

The current results show, that chemosensory stress signals modify social behavior, making women less trustful towards an unknown partner in a social interaction.

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P30- Correlation between olfactory performance and cognitive ability in relation to age in a wide age range of healthy Italian subjects

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Background: Some works showed a reduction of olfactory function within mild cognitive impairment (MCI) subjects (1,2). These studies applied different methods not often comparable or they were performed on small cohorts. In addition, a recent study evaluated olfactory function in MCI patients and healthy controls focusing on elderly subjects with an age range of 65–89 years (3). The aim of our study is to evaluate the correlation between olfactory function and cognitive ability in a wide age range of healthy Italian subjects.
Materials and methods: One hundred and eight participants (age range:20–85 years) were recruited. Olfaction was assessed by means of the Sniffin' Sticks Extended test and cognitive performance was evaluated by means of the Montreal Cognitive Assessment (MoCA) scale (4). Participants were divided into three age groups: 16–35 years (A, n=45), 36–55 years (B, n=21), and > 55 years (C, n=42) (5).
Results: In A age group we observed a significant positive correlation between odor threshold (OT) versus executive function (r=0.366, P<0.05). In B age group we detected a significant positive correlation between TDI score versus memory (r=0.494, P<0.05), between odor discrimination (OD) versus MoCA total score (r=0.564, P≤0.001) and between OD versus memory (r=0.502, P<0.05). In C age group MoCA total score was positively correlated to OD (r=0.360, P<0.05), OI (r=0.499, P<0.001) and TDI score (r=0.550, P<0.001). Moreover, in C age group we noted a positive correlation among executive function versus OD (r=0.318, P<0.05) and versus TDI score (r=0.456, P<0.05).
Conclusion: Our results indicate that a comprehensive olfactory assessment could be considered as biomarker for an early detection of cognitive impairment in subjects with more than 55 years.
References: 1) Conti et al. 2013. 2) Fullard et al. 2016. 3) Tonacci et al. 2017. 4) Conti et al. 2015. 5) Hummel et al. 2007.

P31- Parallel pathways of oscillatory entrainment in mitral cells in the murine accessory olfactory bulb

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The accessory olfactory bulb (AOB) represents the first stage of information processing in the rodent accessory olfactory system. In the AOB, mitral cells (MCs) – the network's sole projection neurons, receive sensory input from peripheral vomeronasal neurons. This sensory information is (pre-)processed in the AOB and relayed to third-order nuclei in the amygdala and hypothalamus. Despite their physiological importance for information processing, the role of MCs in information coding and signal integration is poorly understood.

Recently, we identified a subpopulation of spontaneously oscillating MCs in the mouse AOB. Using voltage- and current-clamp whole-cell recordings in acute AOB tissue slices, we observed both intrinsic rhythmogenesis and network-dependent oscillations.

Intriguingly, we found two independent parallel sources of excitatory synaptic drive that each entrain a distinct subpopulation of oscillating MCs – one dependent on glutamatergic input, whereas the other appears insensitive to pharmacological inhibition of glutamatergic transmission. In ongoing patch-clamp experiments, we now aim to shed light on this non-glutamatergic excitatory drive and to identify the underlying network mechanisms within the AOB.

P32- Different Responses of Fragrance depending on mild and severe Menopause symptoms in Women

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Menopause is the critical periods of women. In this periods, women undergo rapid hormonal change and stop menstruation. Between late 40's to early 50's, most women cannot avoid the menopause. When menopause transition starts, women suffer from physical and emotional symptoms such as hot flashes, night sweat, insomnia, anxiety, mood swings and depressive moods.

To be getting better these symptoms hormone replacement therapy and medications have been used however it has some potential side effects including breast cancer and stroke. Because of this concern, alternative approaches also have been tried to improve symptoms of menopause. Fragrance of essential oils have been reported about many effects such as releasing stress, improving depressive moods and relieving anxiety. These effects are related with menopausal symptoms. It can be helpful for menopausal women and it's easy to use. Despite of these advantages, there are only few studies about its physiological and emotional effects in menopausal women. Furthermore, we need to expand our understanding of menopausal women's odor perception to use fragrance properly and pleasantly. Preliminary studies reported that odor perception was changed during pregnancy or menstrual cycle. Menopausal period also need to check odor perception changes.

Therefore, in this study, we confirmed relax effects of fragrances in menopause women. In addition, we checked physiological and odor perceptional responses of fragrance depending on the mild and severe symptoms of menopause. Mid-life women who are age in 45–55 were attend to this study. The Kupperman index which is widely used for checking seriousness of menopause symptom was used to divide into mild and severe group. Fragrance was chosen from

essential oils by using pre-fragrance estimation. Fragrance L, P, O29 was used in this study.

In consequence, first, mild and severe symptom of menopause group answered differently in fragrance preference and relaxation estimate. Second, the results of physiology and EEG showed similar pattern with fragrance estimate. Specifically, P has more relaxation effect in severe symptom group than mild symptom group. L was effective in mild symptom group. And new fragrance O 29 was effective both mild and severe symptom groups.

Through this study, we notice that response and effects of fragrance may differ depending on severity of symptoms in menopause women. And we confirmed relax effects of fragrances L, P, O after stressful stimulus for menopausal women. Therefore, when we use fragrances to help menopausal women who are suffering from symptoms, we could choose suitable fragrance according to their symptoms of menopause.

Key words: odor perception, menopause symptom, physiology, fragrance estimate, relaxation

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P33- A systematic comparison of semiochemical signaling in the accessory olfactory system of wild and lab strain mice

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In mice, urine is a rich source of chemosensory signals that are essential for con- and heterospecific communication. These cues provide information about social, sexual and reproductive status and regulate complex social behaviors. However, little is known about to what extent these signals vary between mouse strains and, in particular, between wild and typical inbred laboratory mice. In this study, we conduct a systematic comparison of chemosensory responses to urinary signals derived from wild versus inbred laboratory ('lab') strain animals. Parallel imaging and electrophysiological recordings from neurons in both the vomeronasal organ (VNO) and the accessory olfactory bulb (AOB) allow comparative analysis of signal detection and information processing along the accessory olfactory pathway. Large scale Ca^{2+} imaging in acute mouse VNO slices compared neural responses to sex (male versus female) and strain (C57BL/6 versus BALB/c versus wild) specific urinary signals both on the individual neuron and the population level. In addition, in vivo single- and multi-unit recordings from AOB mitral cells in anesthetized mice provide insight into how vomeronasal neuron activity in response to these different stimuli is encoded by the brain.

P34- Mental Whisking - respiratory phase shapes Human task performance and its neural underpinnings

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Terrestrial mammals rely on chemosensory sampling through rhythmic nasal inhalation, namely sniffing. Olfaction, however, is not the only sense orchestrated by the respiratory cycle, and respiratory phase-locking of diverse active sensing mechanisms is common across species. We set out to ask whether respiratory phase impacts human cognition.

We measured nasal airflow during a lexical decision task (n=18, 7F). We observed that individuals had their own highly consistent pattern of phase-locking nasal respiration to task performance, such that mentation was robustly linked with either inhalation or exhalation within an individual (ANOVA intra-subject corr. Fisher-corrected: ISI vs. Preparation vs. Task: $F(2,34)=10.7$, $p<0.001$).

To probe this implicit respiratory preference we designed two follow-up experiments using EEG-ERP (n=25, 7F) and event-related fMRI (n=22, 8F). Unbeknownst to participants, we used their respiratory trace to trigger trials phase-locked to either inhale or exhale onset. In addition to the lexical decision task we applied a spatial task in the EEG experiment and a face-memory task in the fMRI experiment.

Results from EEG-ERP indicate that significantly better performance for trials locked to nasal inhalation in the spatial task (accuracy: IN=73.4 ± 9.1, EX=67.1 ± 8.9, paired t-test $t(25)=-5.02$, $p<0.001$) which was associated with an increased negative component at occipital focus (topographic ANOVA, 180-300ms post stimulus, t-min at O2 = -4.21, $p=0.003$)

Using fMRI, a VOI analysis applied to a contrast of trials presented during inhalation and exhalation revealed a significant increase in BOLD signal associated with nasal inhalation in the left IFG, left thalamus and putamen (ANOVA on BOLD AUC: VOI x Respiration: $F(8,192)=3.89$, $p<0.001$).

Taken together, our results suggest that human cognition is impacted by respiratory phase, and that inhalation may be associated with improved acquisition and processing of information.

P35- OR37 ligand exposure reduces cFos expression in the pvn of the hypothalamus, following open field exposure, without affecting behavioural measures of anxiety

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Members of the OR37 class of olfactory receptors are unusual in projecting, via the MOB, directly to the paraventricular

nucleus (PVN) of the hypothalamus. Previous research has shown that a mixture of three OR37 ligands: pentadecanal, hexadecanal, and heptadecanal, were effective in reducing cFos expression in PVN neurons following novel cage exposure. This suggests that these OR37 ligands potentially have a social buffering effect to reduce stress responses. This study aimed to replicate the original findings of inhibition of cFos expression in the PVN using an open field test. Preliminary results were consistent with the previous finding of inhibition of c-Fos expressions in PVN following exposure to the stressor. However, exposure to the mixture of OR37 ligands failed to have any effect on open field behavioural measures, suggesting a possible dissociation of the effects of OR37 ligands on behavioural and endocrine responses.

P36- The role of umami in the traditional Japanese diet and its potential contribution in other healthy dietary patterns around the world

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With the growing prevalence of overweight, obesity and malnutrition, interventions with healthy diets have become increasingly relevant. Dietary patterns are involved with health positive outcomes such as longevity and lower risk for non-communicable diseases. The Mediterranean diet or the Dietary Approaches to Stop Hypertension (DASH diet) are widely recognized as healthy. They share many features such as high consumption of fresh fruits and vegetables, whole grains and fish, and low intake of red meats. Recent studies have shown that people in Japan who follows closely the Japanese Food Guide Spinning Top has a lower risk of total mortality. The Japanese Food Guide Spinning Top is based on the Japanese traditional diet also known as Washoku. This traditional diet is low in caloric density –low in animal fat and high in water-, nutritionally balanced, and consists on a staple food –cooked rice- that has a plain taste, accompanied with soup, main dish –often grilled fished-, and various side dishes of vegetables and pickles. One of the unique features of Washoku is the mixture of different foods in the mouth where the harmony of overall tastes is achieved. This is possible because the foundation for the flavor of Washoku comes from the dashi stock. Soups and most side dishes are prepared based on the dashi stock and other food ingredients that are rich in umami such as fermented foods and seasonings. It is thought that the skillful use of umami taste is the reason why the traditional Japanese cuisine gives a satisfying meat-like flavor without having to depend on using read meat. We believed that by applying part of the basic concept of the Japanese traditional diet, one soup and three dishes, as a habit together with the strategic use of umami taste in already well-accepted healthy diets, it could be possible to improve the adherence to the diets through a satisfying flavor and even make them healthier by supporting low salt and low caloric-dense dishes.

P37- Chemosensory stress signals augment aggressive behavior in women

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Chemosensory stress signals affect basic social perception and motor behavior in humans. Whether or not effects of chemosensory stress signals extend to complex social behavior is currently unknown. Aim of the current study is to examine the effect of chemosensory stress signals on aggressive behavior. A total of 40 women participated in a modified Hot Sauce Paradigm during which the participants first earned money, and then were frustrated by fictional opponents stealing up to 80% of that money. Participants could penalize their opponent by administering 1 to 10 drops of a hot tabasco sauce. Before deciding on the amount of punishment (hot sauce), participants were exposed to either a chemosensory stress signal, a sport control odor, or pure cotton pad for 3 seconds via a 3-channel-olfactometer with 45 ml/s. The participants were made to believe, they were smelling the body odors of their respective opponents. The chemosensory stress signal was collected via cotton pads from the armpits of 22 healthy men, participating in a modified version of the Trier Social Stress Test for groups. The sport control odor was body odor of the same men exercising on an ergometer. Donors felt more anxious ($p < .001$) and had a higher cortisol level ($p = .001$) in the stress- compared to the control-session. For stimulus administration, cotton pads were pooled across all donors, separately for each condition. As a third stimulus, pure, non-odorous cotton was presented.

After inhaling the stress signal as well as the sport control odor participants administered more hot sauce to their opponent compared to pure cotton (stress: $p = .001$, sport: $p = .017$). The amount of drops was the highest in the stress-condition ($M = 4.1$, $SD = 3.2$) followed by the sport control condition ($M = 3.4$, $SD = 2.9$, $p = .058$). Participants administered the fewest amount of hot sauce after inhaling pure cotton odor ($M = 2.5$, $SD = 2.5$).

The results indicate that chemosensory stress signals affect aggressive behavior in women. These results are of high significance for broadening our knowledge of the evolutional relevance of chemosensory communication.

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P38- Heterogeneity in the Drosophila gustatory receptor complexes that detect aversive compounds

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Animals must detect aversive compounds to survive. Bitter taste neurons express heterogeneous combinations of bitter

receptors that diversify their response profiles, but this remains poorly understood. Here, we describe groups of taste neurons in Drosophila that detect the same bitter compounds using unique combinations of gustatory receptors (GRs). These distinct complexes also confer responsiveness to non-overlapping sets of additional compounds. While either GR32a/GR59c/GR66a or GR22e/GR32a/GR66a heteromultimers are sufficient for lobeline, berberine, and denatonium detection, only GR22e/GR32a/GR66a responds to strychnine. Thus, despite minimal sequence-similarity, Gr22e and Gr59c show considerable but incomplete functional overlap. Since the gain- or loss-of-function of Gr22e or Gr59c alters bitter taste response profiles, we conclude a taste neuron's specific combination of Grs determines its response profile. We suspect the heterogeneity of Gr expression in Drosophila taste neurons diversifies bitter compound detection, improving animal fitness under changing environmental conditions that present a variety of aversive compounds.

P39- Two-dimensional gas chromatography time-of-flight mass spectrometry-based temporal fingerprints of volatile compounds emitted from domestic cat urine

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Domestic cats – one of the most common territorial animals – share parts of their territories with other cats. They spray their urine around the territories' borders for scent marks. Scent signals have advantages over visual and auditory cues, as they remain in the environment for a long time, even if the owners are not present. Scent signals help avoid meeting other cats, which could lead to fighting and injury. Previous studies suggested that cats could distinguish species, sexes, and individuals of scent owners by sniffing the scent marks. However, little is known how chemical compositions of urinary volatile compounds change by time passing, and also whether cats can distinguish the chemical differences of fresh/aging urine between individuals. In the present study, we examined temporal changes of chemical profiles of urinary volatile compounds of domestic cats, and their olfactory discrimination ability toward these volatiles. The headspace gas (400 ml) emitted from the cotton infiltrated with 2 ml male cat urine before and after air-drying were analyzed in two-dimensional gas chromatography (GCxGC)-mass spectrometry (MS). GCxGC-MS detected over two thousands of volatiles in the headspace gas. Statistical analyses indicated that chemical profiles of urinary volatiles were priory divided into two groups, fresh and aging, then into individuals. It suggests the differences caused by temporal changes are more significant than that of individuals. To evaluate cat's discrimination ability, we carried out habituation-dishabituation tests using fresh, 3 h-aging, and 24 h-aging urine samples. The tests showed that cats distinguished the individual differences in fresh and 3 h-aging urine samples, but not in 24 h-aging urine. These findings will improve our understanding of scent signals emitted from excretions in cats.

P40- Peppermint Ambient Odor Affects Task Performance and Cortisol Secretion in Secondary School Children

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There is a growing set of evidences that ambient odors of essential oils have various effects on human mood, behavior and physiological state. A very little published about the impact of odors on behavior and physiology of children though it is known that children surpass adults in the ability to detect and recognize odors, describe their perceptions and preferences. The aim of our study: to evaluate the effect of low concentrations (~ 0.13 mg / m^3) of peppermint oil (Sigma-Aldrich) on the performance of school - children (age 10–11) in the course of ordinary lessons and investigate possible mechanisms underlying the effects. Among different school tests we selected arithmetic dictation test which mainly relies on memory retrieval process. During 15 minutes the teacher dictates with pauses 8 short math exercises, adapted to the student's grade. Students write down only the answers in the corresponding order. Each experiment was set as a series of control tests with no odor alternated with tests accompanied by exposures to peppermint oil. We recorded number of errors and self-corrections. Saliva cortisol was monitored using an ELISA technique (EIA Can-C-290, DBC). Individual saliva samples were taken every 15 minutes during control lesson (no odor, 45 min) and during experimental lesson (peppermint, 45 min). For the reference we also collected in identical manner individual saliva samples from adult students (age 18–21 years, n=20). All samples were kept frozen (-30°C) until analyzed. All experiments were performed at the same time of the day Peppermint improved performance in children (age 10–11) in arithmetic dictation test, causing a significant decrease in number of mistakes as well as in number of self-corrections. This effect was highly significant ($p < 0.001$, n=51) and confirmed by observations in different independent groups: students of 5-th grade ($p < 0.001$, n= 25) and students of 6-th grade ($p < 0.001$, n=30) as well as by repeated observations in the same group (n=3). In our previous research (Rodionova, Minor, 2017) peppermint odor also improved performance of children (8–9 years old) in another test - word dictation but not in text copying test. Word dictation and arithmetic dictation tests depend both on memory retrieval process while text copying test relies mostly on the attention. Saliva cortisol was significantly lowered by peppermint odor in children of both sexes (p=0.00098, n=14)

while in adults we observed significant drop of cortisole only in female students (p=0.00691, n=10). In adult male students we did not observe changes of cortisole secretion under peppermint exposure (p=0.028428, n=10). Stressful events are very common in educational settings. Stress markedly impairs memory retrieval (Vogel, Schwabe, 2016). We hypothesize that peppermint odor may facilitate memory retrieval by lowering cortisole which results in improved performance in word dictation and arithmetic dictation tests.

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P41- Analyses of taste information pathways for fatty acids by neural and behavioral response measurements in mice.

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To investigate potential taste information pathway(s) for fatty acids, we first looked at single fiber responses of the chorda tympani nerve to various taste stimuli including fatty acids in wild type (WT) mice. Among all single fibers tested, 14.3% of fibers showed maximal response to oleic acid or linoleic acid (F-type). Significant responses to fatty acids were also detected in a subset of fibers best responded to sucrose, MPG or $CaCl_2$. In GPR120-KO mice, F-type fiber was 2.0 % in all fiber recorded, and the responses to fatty acids in S-, M- and Ca-type fibers significantly smaller than those in WT mice. Next we looked at behavioral responses by measuring numbers of licks for 10s for oleic acid or linoleic acid mixed with QHCl in WT and GPR120-KO mice. Numbers of licks for these mixtures were significantly smaller in the KO mice than those in WT mice. These results suggest that the specific neural information for fatty acids is carried by F-type fibers, and the nonspecific neural information of fatty acid is carried by subsets of S-, M- and Ca-type fibers. Information from these pathways underlies behavioral responses to fatty acids.

P42- The change of social interaction responses depending on oxytocin in the menopausal women

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Menopause is that every woman will process and the menopausal women experience several symptoms. In this period, the decreasing of oxytocin is related to the decreasing of estrogen which is one of the sex hormones. Oxytocin is related to anti-depression effect, enhancement of social interaction. Especially, according to previous researches, people that have problems with social interaction couldn't contact eyes long time. But, treating the oxytocin, the duration time of eye fixation was improved. Thus, this study focused on how the social interaction is improved when the menopausal women are treated oxytocin.

Participants are recruited 39 women (45–55 age) and an eye-tracker is used to measure the duration time of eye fixation. The oxytocin was treated 3 pumps in each nostril by the nose spray. Then, experiments are processed after 30 mins because it needs to time when the oxytocin reach the brain.

We divided the high Relationship Change Scale(RCS) score group and low RCS score group through social interaction questionnaire results. Interestingly, the duration time of eye fixation percent (%) in low RCS score group was increased in the oxytocin condition compared with the placebo condition. That is, it suggests that the social interaction of women with a low RCS score is improved when the oxytocin is treated to the women.

Based on this study, oxytocin may be helpful to improve social interaction in the menopausal women.

Keywords: oxytocin, social interaction, menopausal women, eye tracker, nose spray

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P43- Spontaneous rapid odor source localization behavior requires interhemispheric communication

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Rodents rely on the ability to identify and spatially localize airborne chemicals to find food, flee from danger or engage in proper social interactions. When rodents monitor the olfactory environment they spontaneously engage in active olfactory sampling behavior, also referred to as exploratory sniffing. Exploratory sniffing is characterized by stereotypical high-frequency respiration, which is also reliably evoked by novel odorant stimuli. To study novelty-induced exploratory sniffing, we developed a novel, non-contact, infrared based method for measuring respiration in a behavioral paradigm in which novel and familiar stimuli are presented to head-restrained mice. We validated the method by simultaneous pressure measurements and confirmed highly reliable detection of inhalation onsets. We further discovered that mice actively orient their nostrils towards novel, previously unexperienced, smells. In line with the speed of olfactory processing reported previously, we find that mice initiate their response already within the first sniff after odor onset. Moreover, bisecting the anterior commissure (AC) disrupted

orienting, indicating the orienting response requires an interhemispheric transfer of information. This suggests mice compare odorant information obtained from the two bilaterally symmetric nostrils to locate the source of the novel odorant. We further demonstrate that asymmetric activation of the anterior olfactory nucleus (AON) is both necessary and sufficient for eliciting orienting responses. These findings support the view that the AON plays an important role in the internostril difference comparison underlying rapid odor source localization.

P44- Chemosensitivity of cholinergic urethral brush cells beyond bitter and umami perception

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Introduction:

Recently, we identified a cholinergic polymodal chemosensory cell in the mammalian urethra (urethral brush cell=UBC) functionally expressing the canonical bitter and umami taste transduction signaling cascade (α-gustducin, PLCβ2, TRPM5). Here, we aimed to determine whether UBC are functionally equipped for salty, fatty and sour detection.

Methods:

UBC were isolated from ChAT-eGFP reporter mice (ChAT=choline acetyltransferase) and intracellular [Ca^{2+}] was recorded by confocal laser scanning microscopy. Stimuli were the bitter stimulus denatonium benzoate (25 mM), ATP (0.5 mM) and NaCl (50 mM), and inhibitors and controls included mannitol (1–150 mM; osmolarity control) and amiloride (0.1 mM; ENaC-inhibitor [ENaC=epithelial Na channel]). Expression of potential sour and fatty acid receptors was investigated in urethral tissue sections by immunohistochemistry (sour: PKD1L3; long-chain fatty acids: GPR120) and utilizing appropriate reporter mouse strains (short-chain fatty acids: GPR41, GPR43).

Results:

NaCl evoked increase in intracellular [Ca^{2+}] in about 71% of UBC (N=84). When responses to both NaCl and denatonium were tested, all three possible response patterns occurred in a balanced distribution. Similar frequencies could be observed regarding the response to ATP and NaCl. Interestingly, about 22% of the UBC (N=37) we tested reacted to all three stimuli, and about 90% of UBC responding to denatonium also responded to ATP.

UBC responding to NaCl (50–150 mM) were used for further investigation of the molecular mechanism of salt detection in UBC. Mannitol in comparable concentrations was used as osmolarity control and had no impact on intracellular [Ca^{2+}]. The NaCl-induced increase in intracellular [Ca^{2+}] was blocked by amiloride. Immunofluorescence showed neither PKD1L3- nor GPR120-immunolabeling of UBC and other urethral epithelial cells, despite positive results in lingual taste buds (PKD1L3) and duodenum (GPR120). Likewise, expression of GPR41 and GPR43 was detected in positive controls (intestine), but not in the urethral epithelium.

Discussion:

NaCl evokes calcium responses in UBC, most likely involving ENaC. This feature does not define a new subpopulation of UBC, but rather emphasizes their polymodal character. So far, there is no evidence for sour and fatty acid detection by UBC.

P45- Bacterial Signal Peptide Increases Mucociliary Clearance in Explanted Mouse Trachea by Stimulating Chemosensory Cells and Paracrine Cholinergic Signaling

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Objective: Mucociliary clearance (MC) is a major innate defense mechanism that removes inhaled pathogens from the airways. Previously it was shown that detection of bacteria-associated molecular cues such as quorum sensing molecules stimulate MC by activation of the canonical bitter taste signaling cascade. Formylated signal peptides, which are ubiquitously produced by bacteria, have recently been found to activate innate immune cells via formyl peptide receptors (Fpr). We here investigated whether this novel class of agonists also influences the mucociliary clearance.
Methods: Cilia-driven particle transport speed (PTS), a readout for MC, was studied in wildtype mice (C57Bl6), in mice lacking a functional Fpr3 (FVB/NCrl) and in mouse strains deficient for crucial components of the canonical taste transduction cascade such as Trpm5 (transient receptor potential channel 5) and Plcβ2 (phospholipase $C_{β2}$). The tracheas of these mice were explanted, transferred to a dish, submerged and dynabeads were added on the surface of the mucosa. PTS was visualized by tracking the directed transport of the dynabeads before and after application of various N-formylated bacterial signal peptides. The transcriptome of single tracheal ciliated and brush cells, a cholinergic

chemosensory epithelial cell type, was analyzed by single cell deep sequencing.

Results: 17 from a panel of 18 formyl peptide receptors agonists did not alter PTS. Only the N-formylated bacterial signal peptide FL185 increased PTS from 45 ± 2 to 73 ± 3 μm/s (mean±SEM; p<0.0001; n=18). Deep sequencing of single tracheal epithelial cells showed Fpr expression in both ciliated and brush cells and the presence of TRPM5, PLCβ2 and several chemoreceptors including Tas2rs in brush cells. Specific Fpr1 and Fpr2 inhibitors [cyclosporine H (1 μM) and t-BOC2 (10 μM)] did not reduce the effect (p=0.6673; n=4). It was also conserved in FVB/NCrl mice. Interestingly, the FL185 response was absent in Trpm5 and PLCβ2-deficient mice. Atropine (1 μM), a muscarinic receptor antagonist, significantly diminished the effect of FL185 (p=0.0022; n=7).

Conclusion: A bacterial signal peptide can stimulate cilia-driven mucociliary clearance. The response does not depend on formyl peptide receptors. Instead, it involves typical taste signal transduction elements, which are uniquely coexpressed in tracheal brush cells, and subsequent cholinergic signaling to ciliated cells. Thus, detection of a defined set of bacterial signal peptides by brush cells via a taste-like signal transduction cascade provides a novel defense mechanism against bacteria.

P46- High level of spatial working memory facilitates the performance in odour memory test - a pilot study

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Odour memory refers to a memory of odours or memory related to odours and evoked by them. It can be described as a sum of perceptional odour information and semantic associations to the odour. This field of knowledge is still relatively new and only some of its aspects have been investigated. Among all, little is known about relations between odour memory and other kinds of memory. In a pilot study involving 24 men and 25 women, we examined if high level of spatial working memory facilitates the performance in an odour memory test. Firstly, all participants took part in the Test of Odor Memory (Sniffin' TOM). Secondly, their spatial working memory level was investigated by The Jigsaw Puzzle Test. Additionally, participants were asked to fill up a short questionnaire about life style elements or health problems that can potentially impact odour memory. We analyzed a relation between individual overall score in The Jigsaw Puzzle Test and the number of correct responses in particular parts of TOM test: (1) the recognition; (2) the free identification; and (3) the cued identification part, separately for each sex. Multiple Linear Regression was used to analyze those relations. As a result, men's overall score in the spatial working memory test positively predicted their increased performance in the cued identification part. On the contrary, in women no relation between the above factors was found. In conclusion, our findings provide the first evidence about a possible existence of a link between spatial working memory and odour memory level. We suggest that this relation appears rather in men but not in women. Thereby, in case of men, creating full-size mental images of odours and rotating them in space, seems to be effective in bringing a semantic association. To investigate this issue in more details, we are planning to conduct our study on a bigger sample.

P47- Prolonged Monosodium Glutamate supplementation does not alter chemosensory performance in old mice

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Monosodium glutamate (MSG) is widely used as a flavor enhancer that may improve behavior and nutritional status in the elderly (Toyama et al., Biol. Pharm. Bull. 31:1852—1854, 2008; Tomoe et al., Ann. N.Y. Acad. Sci. 1170:82–86, 2009). To investigate the effects of MSG on age-dependent chemosensation we evaluated chemosensory ability, motor and memory performance in male CD1 mice 16 months old. Three groups were tested: Control (n=17), NaCl (n=19) and MSG (n=20). For two months mice were given both water and bottles containing either water, NaCl (3 g/L) or MSG (10 g/L). Before the treatment and at the end, mice performed different tests and data were analyzed with ANOVA. All groups lose weight at the end of treatment compared to the beginning. Pole test evaluates the sensorimotor function and long-term memory, being repeated for five days before and after treatment. All groups took longer to descend from the pole on the first day, while no differences in performance was detected in the other days. A food-finding olfactory test was performed before and after treatment, with invisible or visible food. All groups showed similar performance. At the end of treatment, each animal was given access to five taste solutions to evaluate taste preference compared to water. The overall taste preference was similar among groups. Under these conditions, no apparent effect was seen in body weight or in sensory-motor performance.

Those results suggest that MSG treatment did not affect gross olfactory search performance and flavors preference, while subtle difference are still under scrutiny.