Chem. Senses 31: A1–A45, 2011 doi:10.1093/chemse/bjr123

ABSTRACTS

Abstracts from the XXI[st] Congress 2011 of European Chemoreception Research Organization, ECRO-2011, 7–10 September 2011, Manchester Conference Centre, Manchester, UK

PLENARY LECTURES

#1 Beyond pheromone myths: resolving pheromones (species-wide signals) and signature mixtures (variable cues for identity)

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As we celebrate the recent 50th anniversary of pheromones (Wyatt, 2009), real questions about mammal pheromones remain (Doty, 2010) despite the many good examples of olfactory influences on reproduction in mammals (Wyatt, 2003). Part of the problem may be a confusion between pheromones and signature mixtures (variable subsets of molecules of an animal's chemical profile which are learnt by other animals, allowing them to distinguish individuals or social insect colonies (≡ mosaic signals (Johnston, 2003)) (Wyatt, 2010). Not all pheromones are detected by the VNO, though many are. Not all pheromones are innate, though this is common to most pheromones. Instead, what distinguishes signature mixtures from pheromones is the combination of learning and the variability of the cues learnt. In contrast to a species-wide pheromone, there is no single signature mixture to find, as signature mixtures are a 'receiver-side' phenomenon - the differences in signature mixtures allow animals to distinguish each other (Wyatt, 2010).

Classic model systems in mammalian reproduction, such as the Bruce effect (Brennan, 2009), are most easily described when the actions of pheromones, common to all males, are distinguished from each male's individual signature mixture. In another mouse example, the major urinary protein (MUP) pheromone, darcin, prompts the learning of the volatile individual signature mixture of the male (Roberts et al., 2010). Pragmatically, pheromones can be a useful concept.

The distinction between pheromones and signature mixtures is likely to apply to all vertebrate olfactory systems, from fish to birds, as well as chemical communication in other parts of the animal kingdom.

www.zoo.ox.ac.uk/group/pheromones

Brennan, PA (2009). Behav Brain Res, 200, 287–294; Doty, RL (2010). *The great pheromone myth.* Johns Hopkins University Press; Johnston, RE (2003) J Mammal 84, 1141–1162; Roberts et al. (2010) BMC Biol, 8, 75;Wyatt, TD (2003). *Pheromones and animal behaviour.* Cambridge University Press; Wyatt, TD (2009) Nature 457, 262–263; Wyatt, TD (2010) J CompPhysiol A 196, 685–700.

#2 Systems genetics of olfaction in Drosophila

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Most organisms depend on chemical signals from their environment for survival and procreation. Chemosensory behavior presents a substrate for adaptive evolution and, from a genetics perspective, is a complex trait determined by multiple segregating genes that are sensitive to the environment. *Drosophila melanogaster* has one of the best characterized chemosensory systems and presents an ideal model system to dissect the genetic basis for olfactory behavior, since both the genetic background and the environment can be precisely controlled. Recently, complete genome sequences have been obtained for 192 inbred lines derived from a natural Raleigh, NC, population and made available as a community resource, known as the Drosophila Genetic Reference Panel (DGRP). Genetic variance is minimal among individuals within each line, but together the DGRP lines preserve much of the genetic variation that existed in the population from which they were derived, providing a treasure trove of naturally occurring sequence variants for studies on the genetic basis of phenotypic variation. Genome-wide association analyses using the DGRP and analyses of whole-genome transcriptional profiles, together with *P*-element insertional mutations and RNAi-mediated inhibition of gene expression, have enabled studies on the functional diversification of odorant binding proteins, their functional dissection and analysis of their relationships to odorant receptors, identification of single nucleotide polymorphisms in *Odorant binding protein* and *Odorant receptor* genes that are associated with variation in olfactory behavior, and identification of genetic networks that are associated with variation in brain circuitry which underlies variation in olfactory perception.

These studies were supported by NIH grants GM059469 and GM045146.

#3 Modelling olfactory transduction: structure and function of insect pheromone binding proteins

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The structure of pheromone binding protein (PBP) has been analysed by X-ray and NMR. It undergoes conformational changes between two forms (A and B) depending on pH and ligand binding. Modelling is based on kinetic data from electrophysiological, radiometrical and biochemical measurements in moths (*Bombyx mori, Antheraea polyphemus*). Within about 3 ms after entering the sensillum lymph, 17% of pheromone (F) is enzymatically degraded while 83% is bound to the PBP and thereby largely protected from enzymatic degradation. Retarded degradation proceeds within minutes, 20,000-fold more slowly than with the free

pheromone. *In vivo* the complex pheromone-PBP (FA) interacts with the receptor molecule. At weak stimulation the half-life of the active complex is 0.8 s due to the postulated pheromone deactivation. Most likely this process – not to be confused with the enzymatical pheromone degradation - is also enzymatically catalysed; it changes the pheromone-PBP transport complex (FB) into a scavenger complex (FB′), possibly by blocking or removing the C-terminal tail of the PBP. The indirectly determined PBP concentration (3.8 mM) is close to direct measurements. The calculated density of receptor molecules within the plasma membrane of the receptor neuron reaches up to 6000 units per μm^2. This is compared with the estimated densities of the sensory-neuron membrane protein (>300 units per μm^2), and of ion channels (>20 units per μm^2, assumed an open channel conductance of 30 pS). The EC_{50} of the model pheromone-PBP complex interacting with the receptor molecules is 6.8 μM, as compared with the EC_{50} = 1.5 μM of bombykol recently determined using heterologous expression of the receptor molecule. The calculated rate constant of association of the pheromone-PBP complex and the receptor is 1/(s μM).

For literature see: Kaissling KE (2009) Olfactory perireceptor and receptor events in moths: a kinetic model revised. J Comp Physiol A 195:895–922

SYMPOSIA

Symposium 1 - Olfactory systems in mammals: from transduction proteins to behavior
Chair: Anna Menini, (SISSA, International School for Advanced Studies, Trieste, Italy)

#4 Chemo- and thermosensory signaling in the Grueneberg ganglion

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The Grueneberg ganglion (GG) - a cluster of neurons in the anterior nasal region - projects axonal processes to the olfactory bulb and expresses the olfactory marker protein (OMP) as well as distinct olfactory receptors, indicating that the GG may serve an olfactory function. Searching for GG-activating odorous cues, by monitoring the activity-dependent expression of the immediate early gene *c-Fos*, we have recently identified a limited set of defined odorants – in particular dimethyl pyrazines - which elicit responses in murine GG neurons. Responsiveness to these odorous substances was confined to a larger subset of GG neurons which is characterized by the expression of the olfactory receptor V2r83, the transmembrane guanylyl cyclase subtype GC-G and the cyclic nucleotide-gated ion channel CNGA3. Experiments with knockout animals disclosed that GC-G and CNGA3 are important for odor-evoked GG responses. In addition to their responsiveness to odorants, it was found that GG neurons are also activated by another environmental stimulus: cool ambient temperatures. Attempts to unravel the relevant signaling mechanisms revealed that almost all V2r83-/GC-G-/CNGA3-positive GG neurons responded to coolness, i.e, the same subset of GG neurons is activated by coolness and the above mentioned odorants. Experiments with GC-G- and CNGA3-deficient mice demonstrated that these elements contribute to coolness-evoked expression of c-Fos in the GG. Sharing common transduction elements such as GC-G and CNGA3, attempts were made to evaluate whether cross-talks between the coolness-induced and the odorant-activated signaling pathway exist in GG neurons. The results indicate that temperature stimuli markedly affect odor-evoked responses in the GG.

This work was supported by the Deutsche Forschungsgemeinschaft.

#5 Ca^{2+} regulations in olfactory receptor neurons

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Olfactory signaling is mediated by a G protein-coupled transduction cascade that leads to adenylyl cyclase III (AC III) activation, opening of a Ca^{2+}-permeable cyclic nucleotide-gated (CNG) channel and gating of an excitatory Ca^{2+}-activated Cl^- conductance. Additional to this excitatory role of Ca^{2+}, Ca^{2+} is also thought to mediate adaptation via negative feedback on the CNG channel and cAMP generation. Thus, Ca^{2+} is integral to both the magnitude and the time course of the odorant-induced response.

We investigated mechanisms of Ca^{2+} homeostasis in mouse olfactory receptor neurons (ORNs) and found that a Na^+-dependent Ca^{2+} exchanger is required for fast response termination by removing ciliary Ca^{2+} and thus allowing the Ca^{2+}-activated Cl^- channel to close. The molecular identity of the exchanger remained unknown until recently when the $Na^+/Ca^{2+}/K^+$ exchanger 4 (NCKX4) was found in a ciliary protein screen (Stephan et al. 2009). Mice engineered to lack NCKX4 show greatly prolonged responses in electroolfactogram and single cell recordings demonstrating that indeed NCKX4 is the exchanger required for rapid response termination. NCKX4 knockout ORNs showed enhanced adaptation under a double-pulse stimulation paradigm, indicating that Ca^{2+} homeostasis is integral to olfactory adaptation. A manifestation of enhanced adaptation was also that action potential generation was greatly reduced during repetitive stimulations in NCKX4 knockout ORNs suggesting that NCKX4 also can have a profound influence on odor detection and olfactory-driven behaviors.

Stephan et al (2009) PNAS, 106, 11776–11781;

This study was supported by the National Institutes of Health grant DC009613 and a Morley Kare Fellowship.

#6 Odorant receptor and circuit formation in the olfactory bulb

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The odorant receptor identity in the sensory neurons determines not only glomerular convergence and function but also specific connections between glomeruli receiving the same sensory inputs, i.e. homologous glomeruli, within each olfactory bulb (Lodovichi et al., 2003, Belluscio et al., 2002). How such specificity of connections is achieved remain to be understood. Electrical activity plays a critical role in shaping neural circuitry, although the type of

electrical activity required for the development of specific synaptic contacts remain a matter of significant debate. Here we address this issue, analyzing the formation of connections between homologous glomeruli in transgenic mice engineered to have very little afferent spontaneous activity due to the overexpression of the inward rectifying potassium channel Kir2.1 (Yu et al., 2005) in the olfactory sensory neurons. Targeting focal tracer injections to the glomerular layer, we demonstrated that the intrabulbar connections are preserved in these mice, but are not exclusively confined to the homologous glomeruli. The link remains larger than in control mice at all the ages tested, from postnatal day 30 to 70, due to the lack of developmental refinement. Expression of the Kir2.1 channel in adults for 4 weeks was able to induce a regression of the intrabulbar link to an unrefined and enlarged status, indicating the lack of a sensitive period. All together these data indicated that spontaneous activity is required to achieve specificity of connectivity between homologous odor columns in the olfactory bulb.

Lodovichi C, Belluscio L, Katz LC (2003) Functional topography of connections linking mirror symmetric maps in the mouse olfactory bulb. Neuron, 38: 265–276.

Belluscio L, Lodovichi C, Feinstein P, Mombaerts P, Katz LC (2002) Odor receptor instructs functional circuitry in the olfactory bulb. Nature: 419–296–300.

Yu R, Power J, Barnea G, O'Donnell S, Brown HEV, Osborne J, Axel R, Gogos JA (2005) Spontaneous neural activity is required for the establishment and maintenance of the olfactory sensory map. Neuron 42: 553–566.

This study was supported by the Armenise-Harvard career developmental award to CL.

#7 TAARs and innate odor-driven mouse behaviors

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Mouse olfactory sensory neurons detect environmental cues using ~1200 odorant receptors and 14 trace amine-associated receptors (TAARs). TAARs are distantly related to receptors for biogenic amine neurotransmitters, like serotonin and dopamine, that regulate various behavioral and emotional states. To determine the roles of TAARs in olfaction, we identified ligands for 14 olfactory TAARs (6 mouse, 7 rat, 1 zebrafish) that were previously orphan receptors, and each is activated by different combinations of volatile amines. Moreover, several TAAR agonists are metabolites found in urine, a rich source of odors that stimulate instinctive behaviors. One of these receptors, TAAR4, selectively detects 2-phenylethylamine, a carnivore odor that triggers hard-wired aversion circuits in the rodent brain (Ferrero et al, 2011). Predator odors and their cognate receptors are of particular interest since predator odors elicit innate fear-like responses in mice, causing stereotyped avoidance behaviors and influencing the production of endocrine stress hormones. Identifying a ligand-receptor interaction that underlies an innate response to a predator odor would provide a valuable tool to study neural circuits associated with behavior. Using a combination of ligand analysis, behavioral analysis, genetics, and neural mapping, we are dissecting every step in TAAR-mediated neural circuits- from the initial detection of odorants to the ultimate production of behaviors.

Ferrero et al (2011) Proc Natl Acad Sci, in press.

This work was supported by a grant from the National Institute On Deafness And Other Communicative Disorders (Award Number R01DC010155).

Symposium 2 - Physiological Roles of Umami Taste Materials.
Chair: Kunio Torii (AJINOMOTO CO., INC, Japan)

#8 What makes umami pleasant? Multimodal convergence, and top-down attention and cognition

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The cortical processing of umami reveals that the pleasantness of umami reflects and is correlated with processing in the secondary taste cortex in the orbitofrontal cortex and tertiary taste cortex in the anterior cingulate cortex, whereas processing in the primary (insular) taste cortex reflects physical properties such as intensity.

However, glutamate (an umami taste stimulus) presented alone as a taste stimulus is not highly pleasant, and does not act synergistically with other tastes (sweet, salt, bitter and sour). When glutamate is given in combination with a consonant, savory odor (vegetable), the resulting flavor, formed by a convergence of the taste and olfactory pathways in the orbitofrontal cortex, can be much more pleasant, and this is reflected in supralinear activations in the orbotofrontal cortex. Glutamate can thus act to enhance the pleasantness of food by combining supra-linearly with consonant odors in cortical areas where the taste and olfactory pathways converge far beyond the receptors.

Top-down cognitive effects onto the orbitofrontal and pregenual cingulate cortex also interact with the taste and flavour of umami to heighten the palatability of umami. Further, top-down selective attention to pleasantness enhances the responses in the orbitofrontal and pregenual cortex processing stream. Attention to intensity enhances the responses in the primary taste (insular) cortex. Attention-based biased activation of affective vs identity processing cortical streams is thus an important principle of cortical processing related to how food stimuli including umami are processed in the brain.

Rolls, E.T. (2009) Functional neuroimaging of umami taste: what makes umami pleasant. American Journal of Clinical Nutrition 90: 803S–814S.

McCabe, C. and Rolls, E.T. (2007) Umami: a delicious flavor formed by convergence of taste and olfactory pathways in the human brain. European Journal of Neuroscience 25: 1855–1864.

Grabenhorst, F. and Rolls, E .T. (2010) Attentional modulation of affective vs sensory processing: functional connectivity and a top down biased activation theory of selective attention. Journal of Neurophysiology 104: 1649–1660.

Grabenhorst, F. and Rolls, E .T. (2011) Value, pleasure, and choice in the ventral prefrontal cortex. Trends in Cognitive Sciences 15: 56–67.

Grabenhorst, F. and Rolls, E .T. (2008) Selective attention to affective value alters how the brain processes taste stimuli. European Journal of Neuroscience 27: 723–729.

Grabenhorst, F., Rolls, E .T. and Bilderbeck, A. (2008) How cognition modulates affective responses to taste and flavor: top-down

influences on the orbitofrontal and pregenual cingulate cortices. Cerebral Cortex 18: 1549–1559.

#9 Glutamate appetite in laboratory mice: The role of genes, taste and postingestive mechanisms

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The most common umami taste substance is L-glutamate, which is often used in a form of monosodium L-glutamate (MSG). In addition to eliciting the attractive umami taste sensation, L-glutamate has multiple other physiological roles. Both taste and postingestive effects of L-glutamate could be influenced by genetic differences (Bachmanov, 2010). Inbred mice from the C57BL/6ByJ (B6) strain drink more MSG in preference tests than mice from the 129P3/J (129) strain. These two strains differ in postingestive metabolism of L-glutamate but not in taste responses to MSG (Bachmanov et al, 2009). To produce a better model for genetic and physiological studies of glutamate appetite, we have intercrossed the B6 and 129 strains and then used selective breeding to produce mouse strains with high and low MSG intake (MSG-High and MSG-Low). After 10 generations of selective breeding, MSG-High mice drink ~4 times more 300 mM MSG than MSG-Low mice, and they display strong preference for this MSG solution. We used these mice to examine behavioral and neural taste responses to amino acids, and other taste stimuli and nutrients, and found that that strain differences in MSG intake most likely depend on postingestive processes involving glutamate. We also used these strains for genetic analyses. Our initial genetic mapping using hybrids between the B6 and 129 strains has shown that several mouse chromosomes harbor genes affecting MSG intake. Genotyping these chromosomes in MSG-High and MSG-Low mice has confirmed and refined these genetic linkages. These studies allow us to better understand physiological roles of L-glutamate.

Bachmanov et al (2009) Am. J. Clin. Nutr. 90(3):756S–763S; Bachmanov (2010) Perfumer & Flavorist, 35 (4), 52–57.

This study was supported by Ajinomoto Amino Acid Research Program and Ajinomoto, Co.

#10 Umami: taste sensation, hedonics, and satiety

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Umami taste, elicited by L-glutamate, improves the sensory qualities of many foods. However, the effects of glutamate on satiety are poorly understood. The effects of monosodium glutamate (MSG) added to chicken soup were investigated in a sample of young women (n=35). Taste materials were: base broth (63kJ); broth with MSG (1.19g) and nucleotides (0.03g); broth with MSG (1.22g), and broth with added fat (681kJ). A double-blinded within-subject design was used, with each subject exposed to 4 broth conditions on 4 separate days. The broths were presented twice, at 9am and 11:15am, for a maximum cumulative does of 2.44g MSG. Motivational ratings were collected every 15 min post ingestion. Test lunch meal was served at noon and plate waste was measured. The addition of MSG did not affect energy intakes. However, hunger ratings measured following the second administration of MSG broth were significantly reduced relative to the control condition. MSG reduced hunger and desire to snack but had no impact on energy intakes at the next meal. In the second study, young men and women (n=34) tasted and rated 12 soups containing 4 different concentrations of NaCl and 3 concentrations of calcium diglutamate (CDG). Taste intensity and hedonic ratings were obtained. Response surface methodology (RSM) was used to determine the hedonic optima for NaCl and CDG. Analyses showed that CDG could partly replace sodium chloride without reducing liking. These studies suggest that glutamate may have some satiating properties and CDG supplementation can reduce sodium while maintaining taste quality.

Supported by a grant from Ajinomoto Inc to the University of Washington.

#11 Taste and visceral information of glutamate signaling control appetite for food, and operation of digestion satiety for foods due to prevention for obesity

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Gustatory and visceral stimulation of food regulates digestion and nutrient utilization. Free glutamate (Glu) release from food induces the umami taste that increases food palatability. Dietary glutamate is the main source of energy for the intestinal mucosal absorption and metabolism, thus, only a trace amount of Glu reaches the general circulation even after the intake of dietary protein and Glu added in foods. In addition to these roles, we demonstrated a unique gastric sensing system for Glu. Glu is the only amino acid that activates rat gastric vagal afferents from the luminal side possibly via metabotropic Glu receptors on mucosal cells. Functional MRI (4.7T) analysis revealed that luminal sensing with 1% Glu (most preferred concentration) in rat stomach activates the medial preoptic area and the dorsomedial hypothalamus, resulting in diet-induced thermogenesis without changes in food intake. Interestingly, rats fed a high fat and high sugar diet with free access to 1% Glu and water showed lower fat deposition, weight gain and blood leptin compared with those without Glu. From these results, we propose that dietary glutamate functions as a signal for the regulation of gastrointestinal tract via gut-brain axis, contributing to the maintenance of normal appetite for food due to our healthy dietary life.

Symposium 3 - Arthropod chemoreception
Chairs: Matthew Cobb (The University of Manchester, UK) and Marcus Stensmyr (Max Planck Institute for Chemical Ecology, Germany)

#12 Olfaction in two scarab beetles, *Pachnoda interrupta* and *Pachnoda marginata*

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To address how olfactory receptor neuron (ORN) assemblies are adapted to different environments, we screened ORNs using single sensillum recordings (SSR) in two congeneric scarab beetles, *Pachnoda interrupta* Olivier and *Pachnoda marginata* Drury (Coleoptera: Scarabaeidae: Cetoniinae) (Bengtsson et al, 2011). Both species are opportunistic polyphages, feeding mainly on fruit and flowers, and a wide selection of food-related compounds was tested in SSR. Despite occurring in dissimilar habitats (*P. interrupta* is found in dry savannah, and *P. marginata* in tropical parts of Africa), the two species shared most of the physiological types of ORNs encountered. Differences manifested in ORN class proportions, and only few cases of shifts in response spectra, or unique ORN classes, were observed. Most ORNs were functional specialists, responding to one or few compounds, and 95% of active compounds excited a single ORN class.

#13 G-protein signalling in *Drosophila* contact chemoreception

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Chemoreception, via both olfactory and gustatory sensory modalities, is integral to the survival of an organism, as this allows for the detection of both beneficial and harmful stimuli within the environment. However, little is known regarding the molecular mechanisms by which contact chemoreceptors work in invertebrates. Molecular similarities between olfactory and gustatory receptors suggest that gustatory receptors have a G-protein coupled receptor function, yet in *Drosophila melanogaster*, this has not yet been shown. This study therefore used tip-recording electrophysiology to measure the sucrose response of gustatory receptor neurons (GRNs) in the L-type sensilla of Drosophila labellum. This response was pharmacologically manipulated using the G-protein blockers and activators, in the form of non-hydrolysable GDPβS and GTPγS. Additionally, the phospholipase C (PLC) blocker, U73122, was used to further investigate the role of the G-protein transduction cascade in *Drosophila* taste. Results of the study gave preliminary evidence indicating that G-protein is involved in *Drosophila* gustation.

#14 Expression of the *desat1* gene in neural and non-neural tissues separately affect sex pheromone perception and emission in Drosophila melanogaster

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Animals often use sex pheromones for mate choice and reproduction. As for other signals, the genetic control of the emission and perception of sex pheromones must be tightly co-adapted, and yet we still have no worked out example of how these two aspects interact. Most models suggest that emission and perception rely on separate genetic control. We have identified a *Drosophila melanogaster* gene, *desat1*, that is involved in both the emission and the perception of sex pheromones. To explore the mechanism whereby these two aspects of communication interact, we investigated the relationship between the molecular structure, tissue-specific expression and pheromonal phenotypes of *desat1*. We characterized the five *desat1* transcripts—all of which yielded the same desaturase protein—and constructed transgenes with the different *desat1* promoters. Each promoter was used to target reporter transgenes with either (i) the fluorescent GFP marker to reveal *desat1* tissue expression, or (ii) the *desat1* RNA*i* sequence to determine the effects of genetic downregulation on pheromonal phenotypes. We found that desat1 is expressed in a variety of neural and non-neural tissues most of which are involved in reproductive functions. Our results suggest that distinct *desat1* promoters independently drive the expression in non-neural and in neural cells, such that the emission and perception of sex pheromones are precisely coordinated in this species. This finding suggests that desaturase genes are fast-evolving genes which could contribute to the origin of new species and resolves a long-standing enigma in the evolution and function of communication systems.

FB, BH, IC, SC, SD and JFF were supported by the Centre National de la Recherche Scientifique, the Burgundy Regional Council and by the ANR (INSAVEL), TN was supported by Institutional Program for Young Reseacher Overseas Visits from JSPS, DY by MEXT grant 1802012, the Tohoku Neuroscience GCOE program (MEXT), DY and JFF by the Strategic Japanese-French Cooperative Program (CNRS-JST; Structure & Function of Biomolecules).

#15 Crustacean sex pheromones

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The coordination of reproductive behaviour in many crustaceans is critical as the window of opportunity to mate is limited to the female moult during the short reproductive season. Shore crabs, Carcinus maenas and peppermint shrimp, Lysmata bogessi use female produced sex pheromones to attract males and to induce the mating stance (mate guarding) that culminates in mating. Here we describe the identification of two distinct types of crustacean female sex pheromones in these species: the water soluble distance cues that attract mates and induce guarding and the contact sex pheromones that are exoskeleton bound and induce mating stance and mating behaviour. We found nucleotides such as Uridine-di-phosphate to attract males and liphilic cuticular hydrocarbons (CHC's) as these cues. The complexity of the pheromone bouquets used enables species specificity and reproductive isolation and we discuss the implications of the use of simple, reaction specific cues in crustaceans.

#16 Reception of fly odours in *Drosophila*

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Mating is the outcome of successful courtship, and courtship depends on communication between males and females. Although it is well established that Diptera such as flies and mosquitoes use

their sense of smell to locate food sources, the role of odours in their sexual communication is less understood. The fruit fly *Drosophila melanogaster* is experimentally tractable and we use it initially as a model to understand principles underlying courtship that may also operate in medically and economically important Diptera. Using a new stimulus method, whereby the stimulus is loaded onto a microcapillary that is brought into the vicinity of the fly to mimic the proximity of interacting insects, we previously showed that a large proportion (20%) of the fly's olfactory receptor neurons respond specifically to fly odours (Van der Goes van Naters and Carlson, 2007). The differential sensitivity of the neurons to fly extracts and their fractions provides an olfactory basis for the ability of flies to discriminate suitable from unsuitable mating partners. At least four receptors (Or47b, Or65a, Or67d and Or88a) mediate these responses, and we are using genetically encoded light-activated channels targeted to subsets of neurons expressing these receptors to determine how activation of specific subsets of neurons influences courtship behaviour.

Van der Goes van Naters and Carlson (2007) Current Biology, 17, 606–612.

Current research is supported by BBSRC grant BB/H002758.

#17 Specialized noses for specialized lifestyles

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The olfactory system directly interfaces with the environment, thus, changes in the environment, or a change in an animal's habits, as e.g. specialization, would presumably also lead to changes in the olfactory system. Comparative studies on specialized animals, with known generalist ancestors, could hence be a way of revealing general processes shaping olfactory systems, as well as highlighting importance and function of specific chemosensory genes. In this presentation I will outline current work in our laboratory dealing with olfactory adaptations at the molecular, physiological, morphological and behavioral level in a set of highly specialized drosophilid flies.

Symposium 4 - Dissecting the gustatory pathway from taste buds to brain stem.
Chairs: Maik Behrens and Wolfgang Meyerhof (German Institute of Human Nutrition, Germany)

#18 Taste bud cell types and the role of ATP in transmission of taste information

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Stimulation of a taste bud by a tastant activates a variety of transduction systems which culminate in release of ATP from the Type 2 (T1R & T2R-expressing) cells and perhaps from Type 3 (PKD-expressing) cells. The released ATP activates P2X purinergic receptors on the afferent taste nerves to generate the signal communicated to the nucleus of the solitary tract (nTS), the primary gustatory nucleus of the medulla. The extracellular ATP is then degraded by ectoATPase, produced by Type 1 (glial-like) taste cells, to avoid desensitization of the P2X receptors. Genetic deletion of the neural P2X receptors results in "taste-blind" mice where the gustatory nerves fail to respond to all applied tastants (Finger et al 2005). Despite the lack of taste input, these taste-blind mice still respond to ingested substances, presumably via post-ingestive mechanisms or non-taste chemoreceptors in the oral cavity and larynx. For example, the taste-blind mice still avoid strong acids and prefer ingested MSG (Stratford & Finger, 2011) even though these substances do not activate the gustatory nerves. Ingestion of MSG in wildtype mice evokes strong c-Fos activation in the gustatory (rostral) as well as gut (caudal) portions of the nTS. In the KO mice, MSG ingestion only activates the gut region suggesting that MSG detection is entirely post-oral in these taste-blind animals. In contrast, avoidance of citric acid (30mM) by the taste blind mice is intact and evokes a substantial c-Fos signal in both rostral and caudal nTS suggesting oropharyngeal detection of this noxious substance.

Finger et al. (2005) Science 310:1495-1499; Stratford JM, Finger TE (2011) J Neurosci 31:9101–9110.

This study was supported by grants from the Ajinomoto Amino Acid Research Program and by NIDCD.

#19 Detection, modulation and transmission of gustatory sensory signals in the mouse periphery

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Sensory information of taste is crucial for evaluating nutritious and poisonous substances in foods; sweet for carbohydrate sources of calories, salty for minerals, umami for protein and amino acid content, sour for ripeness of fruits and spoiled foods, and bitter for harmful compounds. The detection of these taste qualities begins with receptors on the apical membrane of taste cells. Activation of taste cells leads to depolarization of the taste cell membrane, transmitter release, and activation of gustatory afferent nerve fibers. Considering the coding of taste information, the sensitivity of taste cells and the connection between taste cells and gustatory fibers may be critical in this process.

Recently, we recorded taste responses from mouse fungiform taste bud cells generating action potentials and compared them with those of chorda tympani nerve fibers. We found that both taste cells and fibers fall into several groups with different responsiveness to basic taste stimuli and the occurrence of each group does not significantly differ between taste cells and fibers. We also found that each of sweet-, salt- and umami- responsive types of cells and fibers has similar subtypes segregated by their susceptibilities to receptor inhibitors and antagonists. These data suggest selective connection between corresponding classes of mouse taste cells expressing particular receptors and gustatory nerve fibers, forming coding channels for taste perception. Coding channels for specific taste quality may be devoted to perception of specific taste and those for broad taste qualities may contribute to discrimination of more slight differences between taste compounds.

#20 Dissecting bitter taste

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Bitter taste recognizes countless potential toxins. It involves central neuronal networks that evoke repulsion to prevent food poisoning. However, across taxa, animals actively seek and ingest definite bitter toxins for chemical defense and attack. Moreover, consumers strongly prefer some bitter tasting foods and beverages such as chocolate or coffee containing compounds that exert favorable effects. These observations suggest that bitter taste strongly influences food preferences, and eventually diet and health.

For a detailed understanding of bitter taste physiology and its importance for food selection we first elucidated the response profiles of the TAS2R bitter taste receptors of various species to numerous agonists and provided detailed mechanistic insight into ligand-receptor interactions. Secondly, we examined, in man or mice, the TAS2R repertoire of the oral bitter-responsive receptor cells by *in situ* hybridization as well as genetic labeling and ablation experiments. Thirdly, we characterized bitter TAS2R knockout mice and mice with genetically ablated bitter receptor cells by behavioral experiments and electrophysiological recordings from gustatory nerves. Finally, we studied the functional connection of oral bitter receptor cells with the first order central gustatory neurons in the genetically engineered and control mice by following induction of neural excitation markers.

The results of above experiments will be described and their implication for bitter taste driven rejection of food discussed.

This work was supported by the German Research Foundation (DFG) (ME 1024/2-3) to WM

#21 Genetic tracing of the gustatory neural pathway originating from PKD1L3-expressing type III taste cells in circumvallate and foliate papillae

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Polycystic kidney disease 1-like 3 (PKD1L3) is expressed specifically in sour-sensing type III taste cells that have synaptic contacts with afferent nerve fibers in circumvallate and foliate papillae located in the posterior region of the tongue, though not in fungiform papillae or the palate. To visualize the gustatory neural pathways that originate from type III taste cells in circumvallate and foliate papillae, we established transgenic mouse lines that express the transneuronal tracer wheat germ agglutinin (WGA) under the control of the mouse PKD1L3 gene promoter/enhancer. The WGA transgene was faithfully expressed in *PKD1L3*-expressing type III taste cells in circumvallate and foliate papillae. Punctate WGA protein signals were detected specifically in type III taste cells but not in other types of taste cells. WGA protein was transferred primarily to a subset of neurons located in close proximity to the glossopharyngeal nerve bundles in the nodose/petrosal ganglion. WGA signals were also observed in a small population of neurons in the geniculate ganglion. This result implies the presence of the anatomical connection between taste receptor cells in the foliate papillae and the chorda tympani nerves. WGA protein was further conveyed to neurons in a rostro-central subdivision of the nucleus of the solitary tract. These findings demonstrate that the approximately 10 kb 5'-flanking region of the mouse PKD1L3 gene functions as a type III taste cell-specific promoter/enhancer. In addition, experiments using the pkd1l3-WGA transgenic mice reveal a sour gustatory pathway that originates from taste receptor cells in the posterior region of the tongue (Yamamoto et al, submitted).

This study was supported by Grant-in-Aid for Young Scientists and Scientific Research from the Ministry of Education, Culture, Sports, Science, and Technology of Japan.

#22 Connections of single geniculate ganglion cells: from taste bud to second central synapse

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Taste ganglion cells are functionally heterogeneous. We tested the hypothesis that the ganglion cells are heterogeneous in terms of the central pathways they engage. We used trans-synaptic anterograde pseudorabies virus labeling of fungiform taste papillae to infect single or a few geniculate ganglion cells, together with the central neurons they connect with across two central synapses. The mouse was studied since each bud is specifically innervated by 3-6 ganglion cells; the cells do not branch to multiple buds. Comparisons between cases, revealed "types" of central neural circuits. Some ganglion cells engage, predominantly, the ascending "lemniscal", taste pathway, a circuit associated with higher-order discriminative and homeostatic functions, others engage the "local", intramedullary "reflex" circuit that mediates ingestion and rejection oromotor behaviors. Presumably these differential central connections of the ganglion cells are "hard wired", and relate to the cells' differential responsiveness, e.g., the selective responses of some to molecules representing single taste qualities. In the periphery the taste system is "plastic"; it maintains a critical but little understood relationship between taste bud cells responsive to one taste quality and the ganglion cells signaling that particular quality despite the turnover of receptor cells. To maintain consistent functional signals the ganglion cells must rapidly adapt to the loss of receptor cell inputs by forming connections with new, similar receptors. Should a bud lose, for a time, its entire population of a specific receptor cell type, then ganglion cell terminal fibers could grow to a different bud that does contain that cell type as one mechanism of restoring function. To evaluate such plasticity we hypothesized that ganglion cells innervate different buds over time. Using a series of systematic, sequential bud to ganglion cell dye labeling experiments we established that geniculate ganglion cells do innervate different buds over time, i.e., they can rapidly change the distribution of their peripheral fibers and grow between buds. Such neural redeployment from one bud to another and, presumably, to its receptor cells, could reflect a remodeling mechanism for maintaining consistent receptoneural function and central signaling during receptor cell turnover.

Symposium 5 - The vomeronasal system: from chemosignals to behaviour
Chairs: Peter Brennan (University of Bristol, UK) and Matthieu Keller (CNRS. Nouzilly, France)

#23 Darcin: a male sex pheromone that stimulates attraction and associative learning

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Scents play an integral role in mediating reproductive interactions in many mammals, allowing animals to recognize and locate conspecifics of the opposite sex and to assess the attractiveness of different individuals. Male mice advertise their location and competitive dominance through urinary scent marks deposited around defended territories. These urine marks contain a high concentration of major urinary proteins (MUPs). We show that an atypical male-specific MUP, which we named darcin, elicits the highly repeatable innate sexual attraction of female mice to spend time near male urinary scent marks (Ramm et al, 2008; Roberts et al, 2010). By contrast, females fail to show innate sexual attraction towards other MUPs or to airborne volatiles from male urine. Contact with darcin further stimulates strong and rapid associative learning of the volatiles in an individual male's urinary scent, such that females are subsequently attracted to the airborne urinary odor of that particular male but not to airborne odors of other males. Thus, darcin allows female sexual attraction to be innate but also selective towards individual males. The airborne odor learned is determined in part by the individual-specific pattern of involatile MUPs expressed in a male's urine. These proteins bind low molecular weight hydrophobic urinary volatiles and slow their release from scent marks. Changing an individual male's MUP pattern using recombinant MUPs alters the individual-specific airborne volatile signature that females learn, but a male's urine must contain the pheromone darcin to stimulate such learning. Darcin thus exemplifies a pheromone that can drive the flexible individual-specific social responses that are typical of mammals.

Ramm et al (2008) Proc R Soc B 275, 1727–1735; Roberts et al (2010) BMC Biol 8, 75.

These studies were supported by the Biotechnology and Biological Science Research Council and the Natural Environment Research Council, UK.

#24 Multiple receptor expression in a phylogenetically recent class of vomeronasal neurons

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Mouse vomeronasal type-2 receptors (V2Rs) are restricted to the basal neurons of the VNO and organized in four families. Family-A, B and D (family ABD) V2Rs are expressed monogenically and coexpress with either Vmn2r1 or Vmn2r2, two members of family-C V2Rs. Thus, basal neurons are characterized by specific combinations of two V2Rs.

In this study, we found that six out of seven family-C V2Rs (Vmn2r2-7) largely coexpressed and that none of the anti-Vmn2r2-7 antibodies significantly stained Vmn2r1 positive neurons. Thus, basal neurons are divided into two complementary subsets. The first subset (Vmn2r1-positive) preferentially coexpresses a distinct group of family-ABD V2Rs, whereas the second subset (Vmn2r2-7-positive) coexpresses the remaining group of V2Rs. Phylogenetic reconstruction and the genetic analysis in various species reveal that receptors expressed by this second neuronal subset are recent branches of the V2R tree exclusively present in mouse and rat. Conversely, V2Rs expressed in Vmn2r1 positive neurons, are phylogenetically ancient and found in most vertebrates including rodents. Noticeably, the more recent neuronal subset expresses a type of Major Histocompatibility Complex genes only found in murine species.

These results indicate that the expansion of the V2R repertoire in a murine ancestor occurred with the establishment of a new population of vomeronasal neurons in which coexists the polygenic expression of a recent group of family-C V2Rs (Vmn2r2-7) and the monogenic expression of a recent group of family-ABD V2Rs. This evolutionary innovation could provide a molecular rationale for the exquisite ability in individual recognition and mate choice of murine species.

#25 Chemosensory information processing in the vomeronasal system: from the olfactory bulb to the vomeronasal amygdala

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All living organisms modulate their behavior and physiology in response to external stimuli. In many animals, chemosensory signals from same- and from different species play a particularly important role in this context. The vomeronasal system, by virtue of its ability to mediate such responses, and its relatively compact design, provides an excellent opportunity to study the neuronal substrates that trigger physiological processes upon detection of specific stimuli. My previous experiments revealed that the first brain relay of this system, the accessory olfactory bulb (AOB), maintains a high resolution representation of chemical cues. Thus, the species, sex, and even individual identity of the stimulus donor can be decoded from the activity of neurons. However, a surprising finding was that single units often responded to multiple stimuli that are associated with distinct and even contradicting behavioral implications. How is this potential ambiguity resolved by downstream neurons that ultimately must guide specific outputs? We have begun to address this issue by studying how chemical stimuli are represented in the next processing stage of this system, the vomeronasal amygdala. Comparison of the neurophysiological response properties of the AOB and the vomeronasal amygdala suggests specific hypotheses about the computations that take place between these two processing stages. In my talk, I will describe the results from these recent experiments and their potential implications for the underlying neuronal circuitry.

#26 Involvement of the G-protein Gαo in vomeronasal function and aggressive behavior in mice

Chamero Pablo[1], Katsoulidou Vicky[1], Hendrix Philipp[1], Bufe Bernd[1], Roberts Richard[2], Matsunami Hiroaki[2], Abramowitz Joel[3], Birnbaumer Lutz[3], Zufall Frank[1] and Leinders-Zufall Trese[1]

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The rodent vomeronasal organ (VNO) is specialized in the perception of socially-relevant chemical stimuli and mediates the regulation of species-specific and interspecies social behaviors. The VNO is segregated into two zones containing subpopulations of vomeronasal sensory neurons (VSNs) which differ in their expression of chemosensory receptors and G-protein subunits [either Gnai2 (Gi2) or Gnao1 (Go)]. It is unclear what role the two segregated neural pathways play to control complex social behaviors such as aggression. We used conditional gene targeting to evaluate the role of the Gnao1 gene in a cell-type or organ-specific manner. We employed the Cre-loxP system to delete Go in those cells that express olfactory marker protein, which includes all vomeronasal sensory neurons (VSNs) of the basal layer of the VNO sensory epithelium. As a consequence of the deficiency, the number of VSNs that normally express Go decreased. VSN activation by some peptide and previously identified protein pheromones (Chamero et al, 2007) is drastically reduced in the Go mutants indicating that this G-protein is necessary for the detection of these chemosignals. Detection of other ligands specific for Gi2-expressing VSNs is not affected. Display of both maternal and male territorial aggression is severely diminished. However, unlike mice with genetically ablated VNO function, the Go mutants display a normal mating partner choice and sex behavior. These findings indicate that Go is required in the olfactory system to detect protein and peptide pheromones and is necessary to generate both maternal and male territorial aggressive behavior.

Chamero et al (2007) Nature 450:899–902.

This work was supported by the Deutsche Forschungsgemeinschaft (DFG), the VolkswagenStiftung, and the NIH Intramural Research Program of the NIH.

Symposium 6 - Regulation of odorant and vomeronasal receptor gene expression in mouse
Chair: Peter Mombaerts (Max Planck Institute of Biophysics, Frankfurt, Germany)

#27 Olfaction targeted

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The mammalian olfactory system is increasingly recognized as an attractive model system for studying the formation of neural circuits during development. An olfactory sensory neuron expresses just one the of ~1200 odorant receptor genes in the mouse genome. Axons of neurons that express a given odorant receptor gene coalesce into a few of the ~3600 glomeruli in the olfactory bulb of the mouse. A mapping problem is thus posed: populations of neurons, each expressing a particular odorant receptor gene, must be sorted onto glomeruli. A productive approach to study axonal coalescence is targeted mutagenesis of odorant receptor genes, to express axonal markers in neurons expressing a given odorant receptor gene. These experiments have revealed that the odorant receptor is a critical determinant of axonal coalescence into glomeruli. My talk will summarize recent results of our research on odorant receptor gene choice and axonal wiring.

#28 The versatile guardians of vomeronasal receptor monogenic expression

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In mammals, the perception of semiochemicals by the vomeronasal system is based on the transcription, by each vomeronasal sensory neuron, of a single or a very limited set of chemoreceptor genes. These genes are chosen among large repertoires, that encode for the seven-transmembrane V1R, V2R, and FPR receptors. The mechanisms leading to the control of this strict monogenic and monoallelic expression act apparently both at a global and a more local genetic level. The vomeronasal receptor coding sequence appears to be involved in the allelic exclusion process. Interestingly, this mechanism is apparently shared by non-vomeronasal sensory systems, since swaps of chemoreceptor coding sequences between these systems are able to recapitulate monogenic expression.

#29 Mechanisms governing the expression of the OR37 subfamily of olfactory receptor genes

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In contrast to most other odorant receptor (OR) genes in mammalian species, the members of the OR37 subfamily are expressed by olfactory sensory neurons (OSNs) that are restricted to a small central patch within the olfactory epithelium (OE), raising the question about the control mechanisms governing this special expression pattern. Using bioinformatic approaches, conserved DNA elements upstream of the transcription start sites of all OR37 genes were identified. In mouse lines with a transgene containing the coding sequence and a short upstream region of a representative OR37 gene, its expression was in fact restricted to the characteristic central patch. Comparative bioinformatic analyses of the genomic sequences surrounding the OR37 gene clusters from phylogenetically diverse mammalian species led to the discovery of another short conserved DNA segment positioned between 100 and 400 kb upstream of each OR37 gene cluster, possibly representing a locus control element. Using a transgenic approach in mice which permanently labeled OSNs that ever chose a particular subfamily member (mOR37C) for expression revealed that OSNs broadly dispersed throughout the OE actually select this gene. However, OSNs outside the patch do not maintain it, but finally express an OR gene appropriate for their position. In contrast, in the absence of the olfactory cyclic nucleotide gated ion channel, OSNs continue to express OR37 genes outside the patch. These data indicate that mechanisms acting downstream of gene choice, including odor induced activity shape the expression of the OR37 subfamily.

This work was supported by the Deutsche Forschungsgemeinschaft.

Symposium 7 - Sensory Perception and Language in Eating and Drinking
Chairs: Jeannette Nuessli Guth (ETH Zurich, Switzerland) Larissa Bieler (University of Zurich, Switzerland)

#30 "Eugh": Disgust markers as assessments of food in family mealtime interaction

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Disgust is a theoretical as well as a sensory concept. To be disgusted, one needs to understand not only the sensory characteristics that might be perceived as disgusting, but also the ideational characteristics that mark out 'good' from 'bad', 'right' from 'wrong'. In describing something as disgusting, one is also evaluating it in a particular way. The current paper examines exactly this process: how disgust is used to make an assessment in everyday language use. The topic is food and eating, the setting is family mealtimes. Families in England and Scotland (with English as the native language) were video and audio recorded as they ate meals in their homes. The audio and video data therefore captures naturalistic interaction: as closely as possible to how people would normally act in those settings. A discursive psychological analysis is used to examine the data. This approach focuses on talk as a social practice: the emphasis is on what is achieved socially through talking in a particular way, rather than on individual cognitive processes that may underpin the talk. The analysis focused on instances where speakers uttered a 'disgust marker': such as 'eugh', 'yuck' or 'disgusting'. The analysis demonstrates that disgust markers are typically characterised by three features: they usually follow a 'noticing' about the food/eating practice (i.e. other people's attention is drawn to the target object), they usually occur at the start of a turn in talk (i.e. turn-initial), and they are predominantly uttered alone (i.e. 'eugh') without any explanation or clarification. These features enable disgust markers to make an assessment of food or behaviour without indicating the source of the trouble. That is, whether it is the subject (the consumer) or the object (the food) that is the source of the disgust, is not clear. Disgust markers, in their sequential and intonational detail, are thus argued to blur the subject/object boundary. Speakers may also challenge others' assessments of disgust, and so one can also see the social rights to 'knowing' disgust being managed in talk and interaction.

#31 Taste in twenty cultures

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Scholars disagree about the extent to which language can tell us about conceptualisation of the world. Some believe that language is a direct window onto concepts: Having a word ''bird'', ''table'' or ''sour'' presupposes the corresponding underlying concept, BIRD, TABLE, SOUR. Others disagree. Words are thought to be uninformative, or worse, misleading about our underlying conceptual representations; after all, our mental worlds are full of ideas that we struggle to express in language. How could this be so, argue sceptics, if language were a direct window on our inner life? In this presentation, I consider what language can tell us about the conceptualisation of taste. By considering linguistic data from twenty unrelated cultures – varying in subsistence mode (hunter-gatherer to industrial), ecological zone (rainforest jungle to desert), dwelling type (rural and urban), and so forth – I argue any single language is, indeed, impoverished about what it can reveal about taste. But recurrent lexicalisation patterns across languages can provide valuable insights about human taste experience. Moreover, language patterning is part of the data that a good theory of taste perception has to be answerable for. Taste researchers, therefore, cannot ignore the crosslinguistic facts.

#32 Linguistic features in taste communication

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Language is essential in transferring perceived sensory perceptions. Only verbalization of sensory experiences in eating and drinking allows an exchange on what is perceived. However, as all perceptions in the mouth are individual experiences, meaning might be negotiated in dialogues. This can be observed in everyday life and is for example used in sensory science in a controlled way to standardize a sensory vocabulary for profiling products.

Based on focus group data collected from standard German speakers it is outlined what determines the exchange in talking about taste. Taste has an extended meaning in this case as in language it is referred to overall perception during food consumption. The focus is put on how meaning is created for descriptions of sensory perceptions, especially for taste terms. Examples presented include terms like crispy and crunchy. Linguistic and sensory perspectives are presented to highlight the process of communication in talking about taste and how these findings could be implemented in sensory science.

#33 Iconicity in taste sensation: How body and culture influence language

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Compared to other sensations, taste sensation is genuine in the integration of the perceived object. Indeed, eating or drinking presupposes a physical contact between the perceiver and the perceived object before the integration of a substance. Through various oppositions in terms such as solid vs. liquid, positive vs. negative, filling vs. tasting, iconicity in taste sensation will be pointed as a means to depict how body experience and cultural experience both influence language production. The study of a specific lexicon in English and French through various devices such as morphemic (affixation, compounding), semantic (metaphors, metonymy), or phonological (onomatopoeic expressions) iconicity will attempt to characterize recurrent conceptual motifs in the sense of taste.

#34 The codification of the wine tasting experience. A descriptive algorithm

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This paper is a step forward in the development of a descriptive algorithm used codify the information obtained in the linguistic analysis of wine tasting notes.

The wine tasting restricted lexicon combines two valuable features. It has a very limited number of well defined prototypical referents such as wine, grape, bottle, etc., and the description of the wine tasting experience is highly figurative and mostly metaphorical. Because of this, the description of wines allows for a variety of approaches. And it is this multi-perspectivness what lies at the base of the descriptive algorithm.

A formalism representing this multi source and multisensory experience was needed and it has been developed adopting the format of a descriptive algorithm (DA). Because the type of components needed are highly context and sensory dependent, this algorithm if field-customized and yet standardized.

Based on the structural similarity of the concepts of ontologies, grammars and algorithms, the DA is an extremely simple representational device for describing a lexical item in its context. It is strongly ontologically and lexicographically based and it is easily adaptable to a variety of lexical fields.

Symposium 8 - Odorant-binding proteins
Chair: Paolo Pelosi (University of Pisa, Italy)

#35 Recent advances in understanding vertebrate odorant-binding proteins

Briand Loïc

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Odorant-binding proteins (OBPs) are abundant small soluble proteins secreted in the nasal mucus of a variety of species, from insects to vertebrates including human beings. OBPs reversibly bind odorants with dissociation constants in the micromolar range. OBP binding properties, investigated in rats, demonstrated that the three OBP subtypes are specially tuned towards distinct chemical classes of odorants (Löbel et al, 2002) suggesting a role in odorant discrimination. We have shown that human recombinant variant hOBP-2A is able to bind numerous odorants of different chemical classes with a higher affinity for aldehydes and large fatty acids (Briand et al, 2002). Using site-directed mutagenesis and fluorescent probe displacements, we demonstrated that a single lysyl residue defines the binding specificity of a human OBP for aldehydes (Tcatchoff et al, 2006). Although the physiological function of vertebrate OBPs is not yet clearly established, OBPs are good candidates for carrying airborne odorants, which are commonly hydrophobic molecules, through the aqueous nasal mucus towards olfactory receptors. This carrier role is also supported by their relatively low affinity constants for odorants associated with their high concentration in the olfactory fluids. More recently, we demonstrated a specific OBP-olfactory receptor interaction in vitro that modify the receptor dose-response (Vidic et al, 2008) showing that OBPs may have a more active functional role. We shall discuss the various hypotheses regarding the role of these proteins, as simple transporters or receptor triggers.

Löbel et al (2002) Chem Senses, 27, 39-44; Briand et al (2002) Biochemistry, 41, 7241-52; Tcatchoff et al (2006) FEBS Lett, 580, 2102-8; Vidic et al (2008) Lab Chip, 8, 678-88.

#36 Structural diversity of insects odorant binding proteins Around a conserved core[§].

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[§]- collaborations with the groups of Prof. Paolo Pelosi and Prof. Jing-Jiang Zhou

The discovery in the early 90's of antennal proteins able to bind small volatiles molecules has led to the hypothesis that they may be involved in olfaction and hence have been named Odorant Binding Proteins (OBPs)[1,3]. OBPs have been further subdivides in those able to bind pheromones (Pheromone Binding Proteins (PBPs) and those able to bind general odors General OBPs, GOBPs). The first 3D structure of a PBP[4] and ligand biding studies[5] made it possible to investigate and speculate on ligand binding and specificity of OBPs. It was shown that classical OBPs are composed of six helices and bear 3 disulfide bridges. However, it is only recently that the functional role of OBPs has been assessed[6,7,8]. The advances in genomics allowed another step forward, revealing that insects, such as dipters of hymenopters, possess a very large collection of OBPs from 21 for *Apis mellifera* to 71 for *Anopheles gambiae*. In parallel, bioinformatics and structural biology made it possible to dissect the different structures of classical OBPs in three sub-classes[9]. Furthermore, groups of other OBPs have been identified with only four cysteines (C-minus OBPs) of twelve or more cysteines (C-plus OBPs).

We report here the 3D structures of new classes of OBPs, including a C-plus OBP, a C-minus and an OBP with four disulfide bridges. Based on sequences classification and identity, we have produced an almost complete set of 3D OBP structures for the honeybee *Apis mellifera* and a large number of new structures for *Anopheles gambiae*. The implications of these results in terms of ligand binding will be discussed here.

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#37 Odorant-binding proteins as frontier probes for compounds effecting mosquito olfactory responses

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To identify mosquito repellents of natural origin, an *in vitro* odorant binding protein (OBP) binding assay was employed as screening tool for identification of essential oils containing ligand-like binding activities for specific OBPs of the mosquito malaria vector *Anopheles gambiae*. Binding screens of an initial set of more than 100 essential oils, derived largely from Greek terrestrial plants, against 6 recombinant OBPs expressed at high levels and with a female bias in the antennae of *A. gambiae* revealed the presence of OBP ligand-like compounds in ~40% of the examined oils. By subjecting essential oils containing OBP-binding activities to a behavioral assay employing an artificial warm body, oils containing compounds with mosquito repellent properties were identified. Analysis of the active oils by GC-coupled electroantennography led to the identification of several compounds displaying strong mosquito repellent activities, which could conceivably be developed into control measures for the mosquito vector. To also identify olfactory receptors (ORs) responsible for the recognition of the identified repellent compounds, we employed an insect cell-based OR activity assay that measures photoprotein-mediated luminescence responses occurring upon activation of ORs, ligand-gated cation channels, expressed in lepidopteran cells. The assignment of repellent compound recognition to specific ORs is currently in progress.

This work has been supported by a research grant of the European Union (FP7/2007-2013, ENAROMaTIC project, grant agreement No FP7-222927).

#38 Soluble proteins of chemical communication in vertebrates and insects

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Odorant-binding proteins (OBPs) were the first olfactory proteins, independently identified in 1981 in vertebrates and in insects. Vertebrates OBPs are lipocalins, folded in the conserved structure of b-barrel, while OBPs of insects are mainly composed of a-helical domains. Considered until recently as passive carriers of hydrophobic odorants and pheromones, now strong evidence indicate that in insects OBPs are responsible for detection and recognition of chemical stimuli. Another class of small soluble proteins are involved in insect chemoreception, CSPs (Chemosensory proteins), also structured in a-helical domains, but with a different folding from that of OBPs. CSPs seem to be exclusive of invertebrates and have probably appeared before the insects differentiated from other arthropods, while OBPs exist found only in insects.

All three classes of proteins have also been described in non sensory organs. Often, these proteins are secreted in glands producing biological fluids associated with pheromonal communication. In such districts, OBPs and CSPs are likely involved in solubilising hydrophobic pheromones and helping their delivery in the environment.

In vertebrates, OBPs have been found in urine, saliva, sweat and secretion of the reproductive apparatus. Often they are associated with their endogenous ligands, thus providing a short-cut to the identification of new pheromones.

In insects, recent research indicates a similar phenomenon. OBPs and/or CSPs have been reported in the reproductive organs of Diptera and Orthoptera, as well as in the pheromone glands of Lepidoptera and of the honeybee.

Besides providing an easier way for the identification of pheromones, OBPs involved in the delivery of chemical signals represent an alternative target for the population control of insects of economical importance.

#39 Genome annotation and classification of insect odorant-binding proteins

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Insects mainly use chemical cues (semochemicals) to locate their hosts and the antennae are the main olfaction organ for intercepting the cues and the eliciting of species-specific behaviours. Odorant binding proteins (OBPs) are soluble proteins found in the antennae at very high concentration and believed to function as carriers of semochemicals to the olfactory receptors across the aqueous space between the cuticle and the olfactory receptor membrane. The first OBPs to be identified biochemically were pheromone-binding proteins from lepidopteran species. Later, general odorant-binding proteins were classified, based mainly on their expression in female antennae and non-pheromone responsive sensillum. At the same time another group of proteins, the antennal specific protein (ASPs) were included as OBPs based on the characteristic feature of six highly conserved cysteines and a fixed spacing between them. The chemosensory proteins (CSPs) with four highly conserved cysteines and a fixed spacing are sometime also discussed with OBPs because their specific expression in the antennae. These sequence motifs allowed us to develop an algorithm 'MotifSearch' to search and annotate putative insect OBPs present in insect EST libraries and genome sequences, alongside searches for homologous sequences. Using these approaches we identified OBPs in many insect species and classified them into four subclasses: typical, plus-C, minus-C and CSPs, with most well known OBPs belonging to the typical class. Genome sequence analyses included some OBP-like sequences that are clearly homologous to the OBPs of same species thus are evolutionarily related to OBPs.

Symposium 9 - Odorant ligand based and structure based elucidation of molecular interactions in smell and taste
Chair: Masha Niv (The Hebrew University of Jerusalem, Israel)

#40 The structure and function of odorant receptors: A biophysicist's approach

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Molecular interactions of odorants with their olfactory receptors (ORs) are of central importance for the ability of the mammalian olfactory system to detect and discriminate a vast variety of odours with a limited set of receptors. How a particular OR binds and distinguishes different odorant molecules remains largely unknown on a structural basis. Novel methodologies will be presented for investigating binding of ligands to ORs and subsequent activation of signalling reactions in single cells and in single sub-micrometer sized vesicles with single molecule resolution. Each of these cell-derived vesicles functions as an autonomous container capable to perform cellular signalling reactions. This miniaturization opens novel possibilities for functional screening of receptor-mediated signalling. Examples will be given how screening large odorant libraries on different mutant receptors leads to detailed insight on OR binding sites.

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Etter S, Pick H, Bordoli L, Schmauder R, Schwede T, Vogel H (2009): Dual activities of odorant compounds on olfactory and nuclear hormone receptors. *J. Biol. Chem. 284,* 30547.

Baud O, Etter S, Spreafico M, Bordoli L, Schwede T, Vogel H, Pick H (2011): Mouse eugenol odorant receptor: Structural and functional plasticity of a broadly tuned odorant binding pocket. *Biochemistry 50*, 843.

Thamani D, Vogel H, Pick H (2011): High yield recombinant expression and functional characterization of the mouse olfactory receptor mOR256-17 in mammalian cells.

Biochemistry, dx.doi.org/10.1021/bi2008596|.

#41 Investigation of TAS2R binding sites by functional analyses

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Detection of potentially harmful food components prior to ingestion is achieved by ~25 bitter taste receptors (TAS2Rs) in human. The fact that hundreds of diverse bitter substances are present in nature requires that, on average, each TAS2R interacts with multiple agonists. The ability to detect numerous bitter compounds is not evenly distributed among TAS2Rs resulting in broadly, average, as well as narrowly tuned receptors. As receptor activation involves the establishment of specific contacts between agonists and receptor residues, the requirements on the molecular architecture of binding pockets are complex, particularly for broadly tuned TAS2Rs. To investigate which receptor positions are critical for agonist interaction, we first focused on TAS2R46. From this study we learned that even broadly tuned TAS2Rs possess single ligand binding pockets accommodating diverse agonists by establishing contacts with residues lining a central cavity within the transmembrane domains. Importantly, this and related studies revealed that TAS2Rs, despite low amino acid sequence conservation, exhibit considerable structural similarities with class A GPCRs, making them accessible to molecular modeling approaches. Recently, we began to exploit this fact by investigating TAS2R10, another broadly tuned receptor, by combining functional analyses with molecular modeling. Even though TAS2R46 and -R10 share only ~34% amino acid sequence identity, their agonist activation spectra overlap considerably. Comparing the binding modes of common agonists for both receptors, we conclude that receptor-agonist interaction occurs differently although the binding pockets are positionally conserved. This would argue against an evolutionary conservation of agonist interaction sites originating from common ancestral bitter taste receptors.

This work was supported by the German Research Foundation (DFG) (ME 1024/2-3) to WM

#42 Computational approaches to the study of bitter taste

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Bitter-taste perception in humans is mediated by 25 GPCRs of the hTAS2R gene family. How can merely 25 receptors detect thousands of structurally diverse bitter compounds, and why are some of the receptors broadly-tuned, while others are capable of binding only a small number of ligands?

To elucidate the sites of interaction between the broadly tuned bitter taste receptor hTAS2R14 and its different agonists, we generated an all-atom 3D model of the receptor. Computational docking of two chemically distinct agonists suggests that the binding site is situated inside the trans-membrane bundle and is analogous to previously identified binding pockets of other bitter taste receptors (Brockhoff et al., 2010), and to unrelated GPCRs, summarized in (Levit et al., 2011). Interestingly, different agonists occupy distinct subsites within the same binding pocket, providing a clue to mechanisms of multi-specificity. To facilitate further exploration of bitterness and structure-activity relations of bitter compounds, we introduced a database of bitter compounds, BitterDB (http://bitterdb.agri.huji.ac.il/bitterdb/, (Wiener et al., submitted). This publicly available resource holds over 550 bitter compounds, their structures and chemical properties, as well as known associations with bitter receptors. The database can be searched by structural similarity of compounds and by sequence similarity of receptors. An example of exploring the chemical space of bitter compounds around the known hTAS2R14 agonists is presented as an

illustration of the possible usage scenarios for BitterDB. This resource provides new directions in the identification and design of agonists and antagonists for bitter taste receptors.

Brockhoff A, Behrens M, Niv M.Y, and Meyerhof W. (2010). Structural requirements of bitter taste receptor activation. Proc Natl Acad Sci U S A 107, 11110-11115.

Levit A, Barak D, Behrens M, Meyerhof W, and Niv M.Y. (2011). Homology model-assisted elucidation of binding sites in GPCRs. Methods in Molecular Biology, In press.

Wiener A, Shudler M, Levit A, and Niv M.Y. (submitted). BitterDB, a database of bitter compounds.

This study was supported by the Niedersachsen-Israel research fund and The Hebrew University intramural grant to MYN.

#43 Use of biotech discovery approaches for the identification of potent sweet taste enhancers

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Sweet taste is mediated by an obligatory heterodimeric receptor composed of two subunits called T1R2 and T1R3. These 2 subunits correspond to class C GPCRs characterized by a venus flytrap domain (VFD) at the N-terminus linked to the c-terminal heptahelical transmembrane domain (TMD) via a cystein rich domain (CRD). We used a cell based assay for the human sweet taste receptor, high throughput screening, assay-guided chemical optimization and taste testing with trained panelists to identify positive allosteric modulators (PAMs) that could allow a reduction of the amount of sweeteners used in consumer products. We present here a unique class of PAMs that considerably potentiate sucralose or sucrose effects in the assay but that have no or little agonist activity on their own. In taste tests these PAMs do not have intrinsic sweetness but significantly increase the sweetness of a low amount of sucralose or sucrose. Mutagenesis and molecular modeling suggest that these PAMs act as molecular glue near the opening of the VFT, stabilizing the closed and active conformation via hydrophobic interactions. In consumer product prototypes, these PAMs are significantly more effective than other classical taste enhancers at reducing calories in consumer products without compromising on the true taste of sugar.

#44 Binding of indole induces conformational changes that regulate interactions between odorant binding proteins from *Anopheles gambiae* mosquitoes

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A*nopheles gambiae* mosquitoes use olfactory cues found in human sweat to locate their hosts for a blood meal. The recognition and discrimination of these odors involves the interplay of membrane associated odorant receptors and soluble odorant binding proteins (OBPs) that transport and present odor molecules to the receptors. Recent studies have suggested that recognition of indole, a major attractive component of human sweat, may involve the interactions between two different OBPs, AgOBP-1 and AgOBP-4 (Biessmann *et al.* 2010, Qiao et al 2011). Here we show that the binding of indole to AgOBP-4 induces a major conformational change from a highly dynamic molten globule like state, to a highly ordered conformation and this leads to the formation of a binding site for AgOBP-1. There is no evidence for any interaction between AgOBP-4 and 1 in the absence of ligand. We present a model for how this ligand induced conformational change may regulate the activity of OBPs and we suggest that high levels of intrinsic disorder in some OBPs may represent a common mechanism used to regulating OBP interactions. In additional studies, the crystal structure of AgOBP-1 in the complex with a natural insect repellent, provides new information that can be used to develop reagents to disrupt the normal olfactory responses of these mosquitoes.

Biessmann *et al.* (2010), *PloS One*:e9471; Qiao et al (2011) *Cell. Mol. Life Sci.*, 68, 1799–1813.

This work was funded by National Institute of Health grant R01-DC008834

Symposium 10 - The Role of the Autonomic Nervous System in Central Modification of Sensation
Chair: Peter Julu (Barts and London NHS Trust, UK)

#45 Novel neurophysiological methods for assessing central autonomic activity in real-time: meet the pioneers

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A novel neurophysiological technique has been recently developed for continuous and real-time assessment of brainstem function. Vital signs originating from and regulated by the brainstem are quantified and recorded synchronously for the study of both temporal and causal relationships. These brainstem-regulated vital signs can be classified into two categories: cardiovascular and respiratory. The cardiovascular vital signs quantified and recorded by a machine called the NeuroScope are: Cardiac Vagal Tone (CVT) regulated by nucleus ambiguous in the caudal part of the ventrolateral medulla oblongata, Cardiac Sensitivity to Baroreflex (CSB) regulated in the commissural part of the nucleus of tractus solitarius in the lower part of the dorso-medial medulla oblongata. CSB is under constant hypothalamic influence; therefore it is a good index of supra-medullary influences on autonomic functions. Arterial blood pressure, especially the Mean Arterial blood Pressure (MAP) is regulated in the rostral ventrolateral medulla oblongata and the Heart Rate (HR) is regulated in both the nucleus ambiguous in the lower part (cardiodepressor centre) and the cardioaccelerators are in the rostral part of ventrolateral medulla oblongata. The respiratory vital signs quantified are: Breathing movements, both rate and rhythm are regulated by the ventrolateral brainstem from as low down as the level of first vertebrate to as rostral as the parabrachial nuclei in the ponds and the dorsal respiratory group of neurones in the vicinity of the nucleus of tractus solitarius. Transcutaneous partial pressures of oxygen (pO2) and carbon dioxide (pCO2) representing blood gases are also recorded non-invasively. Blood gases are regulated in the medulla oblongata in conjunction with inputs from the arterial chemoreceptors. These

cardiorespiratory functions of the brainstem are monitored and quantified in real-time and are also time-stamped.

Pioneering Clinical Works: The NeuroScope method can be used to monitor brainstem function in various clinical conditions to study brainstem responses to disease processes or during standardised procedures to examine central autonomic functions. The first clinical breakthrough was in understanding and treating the Cardiorespiratory disorders in Rett syndrome. Intensive research is currently going on to try and understand the central autonomic mechanisms in sensory hypersensitisation in Neurogastroenterology. At the same time, work is also going on to try and study the central modulation of the autonomic nervous system during neuro-immune interactions in patients receiving immuno-therapy due to allergy or substance intolerances. Early results from these studies will be presented in the symposium.

#46 Central autonomic modulation of sensory sensitisation of the human upper gastrointestinal tract

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Introduction: Patients with functional gastrointestinal disorders (FGID) often display persistent and heightened pain sensitivity to experimental gut stimulation, a phenomenon known as visceral pain hypersensitivity (VPH). Current management of pain in FGID involves the use of either antispasmodics or antidepressants; both often give unsatisfactory results. The role of the autonomic nervous system (ANS) in modulating pain is well known, however the effect of ANS modulation on VPH has not been studied.

Aim: to determine whether central modulation of functions of the autonomic nervous system (ANS) using physiological methods can influence the degree of oesophageal VPH in healthy volunteers in a validated model of VPH.

Methods: 35 healthy volunteers (mean age 29yrs; 12 females) underwent psychological profiling for stress, anxiety and personality type. Study 1: Pain thresholds (PT) to proximal oesophageal electrical stimulation (using visual analogue scales) were measured. Thereafter the subjects underwent hydrochloric acid infusion (0.15M) in the distal oesophagus for 30 minutes. This was followed by repeated measurements of oesophageal pain threshold to electrical stimulation in the proximal, unexposed oesophagus at 30, 60 and 90 minutes. Study 2 was performed at an interval of 2 weeks and the protocol was identical to that of study 1 except that during the acid infusion, cardiac vagal tone (CVT) was increased by deep breathing at full inspiratory capacity in 4 sec followed by forced expiratory vital capacity in 6 sec at a frequency of 0.1Hz (6 breaths/minute, over 5 min). The functions of ANS including levels of CVT expressed in units of a Linear Vagal Scale (LVS) were monitored continuously in real time throughout in both studies.

Results: There was only 10% depression and 20% anxiety among our subjects, indicating that our exclusion criteria were effective. The majority of volunteers scored high for 'conscientiousness' and 'neuroticism', which are vulnerability personality factors. 85% of the subjects rated significant for alexithymia. A significant medium correlation, r= 0.35 (p=0.038) was found between strait anxiety levels and the level of PT sensitisation. In study 1; we observed a non-significant reduction in CVT during acid exposure compared to baseline level, the average difference was -0.27 units of LVS, while in study 2 CVT increased significantly during combined acid exposure and deep breathing compared to baseline level and the average difference was 2.05 units of LVS (SE ±0.684, p = 0.005). In study 2, 83% of the volunteers reduced their level of oesophageal sensitisation to acid in comparison to study 1, and 60% of them failed to sensitise completely. Overall, there was a significant reduction in sensitisation to electrical stimulation in the magnitude of 17.61 mA of stimulus strength between study 1 and 2 (SE ±2.384, p = 0.0002). A strong inverse correlation of r= -0.556 (p=0.0006) was detected, between the difference in CVT and the level of PT sensitisation.

Conclusion: This study suggests that central parasympathetic activation has anti-hyperalgesic properties in human oesophageal VPH. This data also suggests a possible change in the way we treat visceral pain. There are potentially new targets for the development of novel treatments of VPH using central modulation of CVT. However, further research is required focusing on exploring the mechanism of sensory desensitisation during central autonomic modulation caused by deep breathing.

#47 Abnormal spontaneous brainstem activity (asbas) following low-dose subcutaneous injections of macromolecular antigens in humans

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Introduction: Abnormal spontaneous brainstem activations (ASBAs) are novel observations in our Autonomic Laboratory in which the brainstem become irritable and its neurones are spontaneously stimulated leading to specific and recognisable cardiovascular events. These are observed in the heart rate, arterial blood pressure, cardiac vagal tone and baroreflex sensitivity records. These anomalous brainstem activations can cause startle responses and in some extreme cases it is associated with screaming. We have observed ASBAs consistently in patients undergoing low dose immunotherapy and we wanted to investigate the effects of low dose immunotherapy on brainstem functions.

Methods: Four consecutive patients undergoing low dose immunotherapy were recruited for live and real-time assessment of brainstem autonomic function in our laboratory while undergoing their routine treatments. They were given subcutaneous injections of diluted whole Baker's yeast in the upper arm. We then used the NeuroScope™ (MediFit Instruments Ltd, London) to record modified Einthoven's Lead II electrocardiogram (ECG) continuously and simultaneously with beat-to-beat arterial blood pressure (BP), breathing movements, arterial blood gases measured transcutaneously, cardiac vagal tone (CVT) and cardiac sensitivity to baroreflex (CSB). Beat-to-beat heart rate (HR) was calculated from the ECG R-R intervals. These cardiovascular data were synchronised and time-stamped in real-time and stored in a computer for later analyses of sequences of events and causal relationships. All the above cardiovascular indices are regulated in various parts of the brainstem and therefore serve as measures of the respective parts of brainstem functions.

Results: All four patients showed ASBAs of various severities with duration between 10-180s. They were characterised by what at first looks like simultaneous ramp increases of variable gradients

in HR, BP, CVT and CSB followed by abrupt decays also of variable gradients. Careful analyses of the sequences of cardiovascular responses revealed that most of the ASBAs started from the lower ventro-lateral medulla oblongata in the cardio-depressor area of the nucleus ambiguous where CVT is regulated and it spread dorsally to the tail of the nucleus of tractus solitarius where CSB is regulated and continued to spread rostrally to involve the rostral ventro-lateral medulla where vasomotor pre-sympathetic neurones regulating BP and cardioaccelerator neurones regulating HR are found. The arrhythmia was seen in the HR record as repetitive ramps of tachycardia ending abruptly in rebound bradycardia. The ECG remained in sinus rhythm throughout the ASBAs and no ventricular abnormality was seen in the ECG. Normal breathing rhythm was maintained and there were no changes in levels of blood gases during these ASBAs.

Conclusion: We have described here the immediate induction of ASBAs in patients given subcutaneous injections of complex macromolecular antigens in the arm. We have also demonstrated the sequence of cardiovascular events in the brainstem during these spontaneous excitations. It was clear that excitation started from the lower brainstem in medulla oblongata and spread rostrally within the brainstem. Repetitive injections of the macromolecular antigens during the on-going immunotherapy established the causal relationships between the antigens and the ASBAs that followed the injections.

#48 Modulation of the central autonomic activity using low-dose subcutaneous injections of macromolecular antigens in humans

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Introduction: Allergy, or abnormal reactivity to foods is now clinically recognised but the definition is still controversial and is still being debated. Emil von Behring introduced the term 'hypersensitivity' to describe the exaggerated response of guinea pigs to repeated doses of diphtheria toxin, but the term is sometimes used to describe reactions to foods. Clemens von Pirquet, defined allergy simply as ''altered reactivity''. Immunological processes are modified by neuro-endocrine systems, which are in turn influenced by external factors. The main neuro-modulator of the immune response is the autonomic nervous system. However, the concentration of the allergens itself also plays a very important role in the modulation of the immune responses and therefore the ultimate allergic reactions. Repetitive administration of the same allergen in either increasing or decreasing doses both appear to abrogate the allergic reaction. This is rather puzzling if considered in terms of a straight forwards orthodox biochemical dose-response curve. John Freeman employed allergen-specific immunotherapy in which increasing doses of allergen were used to force the body to accept these challenges, a process he called desensitisation. Miller used decreasing doses of allergens to achieve a similar effect in a process described as ''Low-Dose Immunotherapy'' and the diluted allergens are referred to as ''vaccines''.

Aims and Hypothesis: Since the immune response is closely linked with the autonomic nervous activity, we hypothesise that allergen-specific immunotherapy modulates central autonomic activity. We therefore investigated the long-term effects of allergen vaccines on the central autonomic functions in patients with previously identified autonomic dysfunctions.

Methods: 13 patients who had the confirmation of combined failures or subnormal skeletal muscle vasoconstrictor functions and cardioaccelerator dysfunctions were selected for the study from our routine allergy clinics. Their written informed consents were obtained for clinical audit and publication purposes. We assessed skeletal muscle vasoconstrictor function by measuring the increase in diastolic blood pressure (ΔDBP) and cardioaccelerator function by measuring the increase in heart rate (ΔHR), measured continuously in real time during standardised isometric contractions of muscles of the dominant hand using the NeuroScope method. Patients were allowed to start their allergy treatment consisting of self-administered daily subcutaneous injections of a cocktail of allergen vaccines immediately following the initial autonomic assessment. Treatment was terminated immediately in cases of intolerance or improvement to the extent that injection was no longer required. Autonomic assessment was repeated following treatment.

Results: Ten patients (77%) reported improvement of their symptoms (Responders) and three patients (23%) reported no improvement in symptoms (Non-responders). There were more than twofold increases in both vasoconstrictor function in skeletal muscles and cardioaccelerator function in the heart in the Responders. ΔDBP was (means ±SEM) 12.7±0.9 and it increased to 32.1±2.6 mmHg following treatment ($p<0.00001$). DHR was 13.3±2.3 % and it increased to 39.8±6.3 % ($p<0.0001$). There were no significant changes in autonomic functions among the Non-responders. ΔDBP was 18.7±4.1 before and 11.3±3.6 mmHg after treatment ($p>0.4$). ΔHR was 19.2±4.1 % before and 13.9±3.2 % after treatment ($p>0.49$).

Conclusion: Since both cardioaccelerator and skeletal muscle vasoconstrictor functions are regulated in the rostral ventro-lateral medulla oblongata, we conclude that repetitive subcutaneous injections of macromolecular allergen vaccines modulates central autonomic activity in the long-term. Our results also suggest that improvement of symptoms may be linked with improvement in central autonomic functions. Therefore the results suggest that low-dose immunotherapy can ameliorate the central autonomic dysregulations triggered by allergy or substance intolerance.

#49 Central autonomic modulation of the perception of nausea in humans

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Introduction: Nausea is a common and complex multi-system subjective symptom. There is a lack of objective biomarkers to assess nausea and nausea susceptibility in humans, which makes clinical decisions difficult. Furthermore, animal studies have no agreed criteria to identify nausea and are associated with moral and animal welfare issues.

Aim: To study the psychophysiological, central autonomic modulations and brain processing responses to a novel human model of nausea.

Methods: A 10-minute video that induced motion sickness and another that did not (control video) were presented to 51 healthy volunteers (age 27 ± 8 years, 22 male). They were tested for nausea using a validated scoring system. Personality questionnaires were used for psychophysiological assessment. Cardiac sympathetic activity was measured using the cardiac sympathetic index (CSI). Other indices of sympathetic activity used were: heart rate (HR) and mean arterial blood pressure (MAP). We monitored central parasympathetic activity by measuring cardiac vagal tone (CVT) and cardiac sensitivity to baroreceptor reflex (CSB). We also measured cortisol levels and electrogastrogram (EGG). Subsequently, 9 subjects (aged 25 ±5 years, 5 males) with proven susceptibility to nausea had the same stimuli again with functional MRI (fMRI) that was analysed with XBAM.

Results: All subjects completed the studies without vomiting. When comparing nausea video (NV) to control, NV raised nausea scores (nausea VAS, +57%±11, p<0.01). NV also raised cardiac sympathetic activity (HR +4.04±0.94, p<0.01; SBP +2.4±1.75, p=0.18; DBP +2.23±0.98, p<0.05; MAP +2.28±1.12, p<0.05) and reduced parasympathetic activity (CVT −1.36±0.49, p<0.01; CSB −1.27±0.61, p<0.05); and raised the dominant frequency on EGG (3.0±0.04 vs 2.8±0.05, p<0.02). When comparing nausea susceptible (NS) with resistant subjects (NR), the NS subjects reduced their CVT more than NR subjects (−20.6±7.7 in NS compared to −11.2±0.8 in NR subjects, % change from baseline, p<0.01) and CSB dropped by −25.1±9.4 in NS compared to −8.2±10.7 % in NR subjects, p<0.05. The HR rose by 10.4±3.4 % in NS compared to 0.8±2.2 % in NR subjects, p<0.05. Cortisol level rose by 16.9±20.2 % in NS compared to a drop by −28.5±4.9 % in NR subjects, p<0.05. The dominant power in the EGG was reduced by −2.81±1.36 % in NS compared to only −0.17±0.93 % in NR subjects, p<0.01. The 9 NS subjects who had fMRI showed correlations to nausea levels with an increase in brain activity in the inferior frontal gyrus (p<0.01) and temporal lobe (p<0.01) and a decrease in brain activity in multiple areas of the occipital lobe (p<0.01) and cerebellum (p<0.05) when the records taken during NV were compared to those taken during the control video.

Conclusion: This human model of nausea is safe and effective and we recommend it as a research tool. NS subjects had central autonomic modulations measured as reduced CVT and CSB, which was the cause of the raised HR. The stress hormone cortisol was also raised suggesting neuro-hormonal interactions and the dominant frequency of the EGG was reduced in power compared with baseline levels more among NS subjects compared with NR subjects. The NS subjects also showed different brain processing patterns during NV compared to control video. Since motion sickness is known to induce gastrointestinal (GI) physiological changes, this human model can be used to study the effects of nausea on GI function in both health and in disease together with the option of correlating the changes with objective physiological and brain processing biomarkers.

#50 Impaired central modulation of autonomic activity in neurodegenerative disorders

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Introduction: Central respiratory neurons in the brainstem have very strong influence on autonomic activity. The well-known effect is respiratory sinus arrhythmia. This is caused mainly by the cholinergic interneurons connecting respiratory neurons to cardiac vagal motor neurons in the brainstem. The baroreceptor signal to the cardiac vagal motor neurones responsible for maintaining cardiac vagal tone (CVT) is mediated by monoaminergic neurones. The NeuroScope method can monitor the activity of cardiac vagal motor neurons in real time. This is achieved by measuring CVT in real time.

Hypothesis and Aims: We hypothesise that the well-documented disturbances of the balance between cholinergic and monoaminergic neurones in neurodegerative disorders extends to the brainstem. We therefore investigated the modulatory effects of respiratory neurons on cardiac vagal motor neurons during deep breathing exercises in healthy controls and in patients with neurodegenerative disorders.

Methods: Non-invasive real time and continuous monitoring of cardiac vagal tone (CVT) was done at baseline in supine position during quiet breathing and during deep breathing exercises in 6 healthy controls, 5 male and 1 female aged 24-40 years to establish the type of central autonomic modulation during deep breathing. Eight patients with neurodegenerative disorders, all were suspected of suffering from multiple system atrophy (MSA) or Parkinson's disease. Four males aged 71-73 and 4 females aged 60-67 years were studied. Eight more patients within these age ranges but without neurodegerative disorders were also studied. They had dysautonomia of other causes. There were 4 males aged 67-74 and 4 females aged 59-69 years in this group. The results were analysed and compared. The CVT was measured using the NeuroScope and expressed in units of a linear vagal scale (LVS). The subjects were in the supine position during the initial rest period and throughout the deep breathing exercises. Each subject was instructed to breathe deeply at 10 seconds intervals during which full inspiration was achieved within 4 seconds followed by forced expirations lasting 6 seconds. The exercise continued for one minute. The local ethics committee of the Imperial College, London, approved this study.

Results: The average CVT over the one-minute period during deep breathing exercises in healthy people was (mean ±S.E.M., units of LVS) 16.4±2.6, a significant increase above the baseline CVT of 9.1±1.6 (P<0.04, paired Student's t-test where P<0.05 is statistically significant). The peak CVT during deep breathing was 25.1±4.0, 276% of the baseline CVT (P < 0.005) indicating significant elevation. The average CVT in similar deep breathing exercise in age matched patients with no neurodegerative disorders was 9.3±1.1, which was a significant rise above a baseline of 3.6±0.6 (p<0.0004) and the peak significantly rose to 14.0±1.8, 389% of the baseline level (p<0.0005). The average CVT during deep breathing was 2.6±0.6 and was not significantly different from the baseline level of 2.3±0.7 (p>0.3), but the peak CVT was 3.7±0.7, it significantly increased to 160% of baseline level in patients with neurodegenerative disorders.

Conclusion: We have shown that the cholinergic dependant central respiratory modulation of cardiac vagal motor neurones is impaired in neurodegenerative disorders. It suggests that cholinergic interneurones may be affected in the lower brainstem in

neurodegerative disorders more than it is currently known. This is new data and requires further investigation in a large study. Our data also suggests that respiratory modulation of CVT may be a useful test to distinguish neurodegenerative disorders from other causes of dysautonomia with similar clinical presentation of abnormally low resting CVT. Such discriminatory test is of high prognostic significance.

#51 Dietary modulation of central parasympathetic activity in neurodevelopmental disorders of the autistic spectrum

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Introduction: Autistic Spectrum Disorder (ASD) is likely to be a heterogeneous group of conditions affecting behaviour. Identifying the sub-groups involved and the pathophysiology of the commonest chronic childhood condition may lead to effective strategies for reducing the impact of the disease, and perhaps even prevention.

In a previous study, low central parasympathetic tone was identified in 80% of ASD children. It is thought the low parasympathetic tone relates to gastrointestinal upsets seen in up to 70% of ASD children.

Case Studies: We present here three case studies highlighting quantifiable improvements of the resting central parasympathetic tone following dietary alterations. The dietary changes also led to neurobehavioural improvement and favourable changes in other baseline autonomic functions. The major central parasympathetic activity quantified was Cardiac Vagal Tone, but signs of brainstem irritability indicated by frequent episodes of Abnormal Spontaneous Brainstem Activations (ASBAs) were also studied.

We shall discuss possible mechanisms involved in ASD and give some insights into what is increasingly being thought of as a neuroimmune disorder.

#52 Central autonomic modulation of the perception of nausea in humans

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Introduction: Nausea is a common and complex multi-system subjective symptom. There is a lack of objective biomarkers to assess nausea and nausea susceptibility in humans, which makes clinical decisions difficult. Furthermore, animal studies have no agreed criteria to identify nausea and are associated with moral and animal welfare issues.

Aim: To study the psychophysiological, central autonomic modulations and brain processing responses to a novel human model of nausea.

Methods: A 10-minute video that induced motion sickness and another that did not (control video) were presented to 51 healthy volunteers (age 27 ± 8 years, 22 male). They were tested for nausea using a validated scoring system. Personality questionnaires were used for psychophysiological assessment. Cardiac sympathetic activity was measured using the cardiac sympathetic index (CSI). Other indices of sympathetic activity used were: heart rate (HR) and mean arterial blood pressure (MAP). We monitored central parasympathetic activity by measuring cardiac vagal tone (CVT) and cardiac sensitivity to baroreceptor reflex (CSB). We also measured cortisol levels and electrogastrogram (EGG). Subsequently, 9 subjects (aged 25 ±5 years, 5 males) with proven susceptibility to nausea had the same stimuli again with functional MRI (fMRI) that was analysed with XBAM.

Results: All subjects completed the studies without vomiting. When comparing nausea video (NV) to control, NV raised nausea scores (nausea VAS, +57%±11, $p<0.01$). NV also raised cardiac sympathetic activity (HR +4.04 ± 0.94, $p<0.01$; SBP +2.4 ± 1.75, $p=0.18$; DBP +2.23±0.98, $p<0.05$; MAP +2.28±1.12, $p<0.05$) and reduced parasympathetic activity (CVT −1.36 ±0.49, $p<0.01$; CSB −1.27±0.61, $p<0.05$); and raised the dominant frequency on EGG (3.0±0.04 vs 2.8±0.05, $p<0.02$). When comparing nausea susceptible (NS) with resistant subjects (NR), the NS subjects reduced their CVT more than NR subjects (−20.6±7.7 in NS compared to −11.2±0.8 in NR subjects, % change from baseline, $p<0.01$) and CSB dropped by −25.1±9.4 in NS compared to −8.2±10.7 % in NR subjects, $p<0.05$. The HR rose by 10.4±3.4 % in NS compared to 0.8±2.2 % in NR subjects, $p<0.05$. Cortisol level rose by 16.9±20.2 % in NS compared to a drop by −28.5±4.9 % in NR subjects, $p<0.05$. The dominant power in the EGG was reduced by −2.81±1.36 % in NS compared to only −0.17±0.93 % in NR subjects, $p<0.01$. The 9 NS subjects who had fMRI showed correlations to nausea levels with an increase in brain activity in the inferior frontal gyrus ($p<0.01$) and temporal lobe ($p<0.01$) and a decrease in brain activity in multiple areas of the occipital lobe ($p<0.01$) and cerebellum ($p<0.05$) when the records taken during NV were compared to those taken during the control video.

Conclusion: This human model of nausea is safe and effective and we recommend it as a research tool. NS subjects had central autonomic modulations measured as reduced CVT and CSB, which was the cause of the raised HR. The stress hormone cortisol was also raised suggesting neuro-hormonal interactions and the dominant frequency of the EGG was reduced in power compared with baseline levels more among NS subjects compared with NR subjects. The NS subjects also showed different brain processing patterns during NV compared to control video. Since motion sickness is known to induce gastrointestinal (GI) physiological changes, this human model can be used to study the effects of nausea on GI function in both health and in disease together with the option of correlating the changes with objective physiological and brain processing biomarkers.

#53 Sensory neural monitoring of immune challenge and neural control of host defence

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This review will present the compelling evidence of direct connections between the neural and immune system. Elements of this

neuroimmune system have been recognised for a long time within different disciplines but treated as isolate and peripheral neuroimmune "collaborations" rather than connections between brain and body. For example the connections between peripheral sensory nerve fibres and epidermal Langerhans cells in the skin have been recognised for a long time by dermatologists who utilise the neural components to treat inflammation. However, to a neuroscientist, this connection forms a powerful sensory system where the brain is informed of challenges to the body through neural monitoring of the antigen processing by the ELCs. The brain has direct nerve connections to the bone marrow and lymph nodes and, through these, elicits the appropriate response.

Disconnecting the neural system from these sites of interaction with the immune system leads to malfunctions and failures. Central brain connections have now been mapped from some of these sites of interactions and point to specific brain regions involved in host monitoring and host defence.

It is highly likely that an understanding of the central circuits involved will allow more precise control of host defence activity, including control of abnormal activity such as allergies, autoimmunity, and bone marrow malfunctions.

Symposium 11 - The influence of gender and sexual orientation on human olfaction
Chairs: Katrin Luebke, Heinrich Heine University of Dusseldorf, Germany, and Mark Sergeant ,Nottingham Trent University, UK

#54 Effects of gender on human chemosensory communication

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Human chemosensory signals facilitate social communication about the genetic profile, the nutritional and the health status, and the level of endocrine activity. However, messages related to neuronal activity can also be conveyed, as chemosensory signals are associated with psychological trait and state variables. E.g., just recently it has been shown that emotional communication in humans is effectively supported by chemosensory signals. As chemosensory communication in humans is often considered to reflect an ancient channel of information transmission, phylogentic and ontogenetic survival might have strongly promoted through chemosensory communication.

It is here stated, that human chemosensory communication adds to social communication in general in a basic and important manner. According to accumulating empirical evidence, women outperform men in many tests related to the encoding and decoding of social signals. Here, it will be reviewed that also the processing of and adjustment to social chemosignals seems to be advantageous in women.

The processing of and adjustment to social chemosignals in humans has been investigated with a number of different bioassays. Hereby, it has been shown that women use chemosignals more effectively as contextual cues while processing visual social signals. Moreover, women demonstrate more efficient pre-attentive stimulus decoding strategies in differentiating social safety from social danger cues. However, motor adjustments to social signals of danger are primed as effectively in women and in men. The reasons for the gender differences in human chemosensory communication will be discussed.

#55 Gender-specific olfactory effects

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In general, the female sense of smell typically outperforms the male sense of smell; however, there are interesting, gender-specific influences on both olfactory perception and production of body odor. Both gender and sexual orientation influence production of and perception of body odor. Body odor from males also influences pulsing of luteinizing hormone and alters mood in women. Whether analogous effects are observed in males exposed to body odor from females remains to be determined. Exposure to an odorant results in adaptation but may also induce cross-adaptation to other odorants. Cross-adaptation can be readily observed with fragrance materials, e.g., many musk materials mutually cross-adapt and the effects are similar in men and women. Impact of agricultural odors can be reduced by pre-exposure to some pleasant odorants, which occurs to the same extent in men and women. Dozens of fragrance materials were employed in cross-adaptation experiments to determine efficacy in reducing impact of human body odor. In the nose of men, a subset of materials was effective at reducing the perception of both male and female body odor; however, none of the materials were effective against male body odor in the noses of women and only two significantly altered perception of female body odor. Body odor can predict underlying immune genes, which may be important information for women potentially selecting a mate. Continued perception of female body odor by women may be critical in assessing potential competition or may be an important determinant in strategies for female-female cooperation.

#56 Effects of sexual orientation on human chemosensory communication

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Sexual orientation influences both preferences for complex human body odors as well as central nervous processing of some of its single molecule compounds.

Here, we present data concerning the effects of sexual orientation on the central nervous processing of complex human body odors obtained from potential partners as opposed to individuals not constituting potential partners in regards to gender and sexual orientation. By means of cotton pads, axillary odours were collected from 49 homosexual and heterosexual men and women and presented to 28 (14 lesbian) women as well as 28 (14 gay) men via a constant-flow olfactometer. The EEG was recorded from 61 scalp positions and chemosensory event-related potentials as well as current source densities (CSDs) were analyzed.

Results show a processing advantage on the level of early stimulus encoding (P2-latency) for body odors obtained from potential partners. On the level of later stimulus evaluation (P3 amplitude),

processing of body odors obtained from individuals not constituting potential partners was enhanced in both gay men and lesbian women, the activity correlating to neuronal sources in medial frontal and parietal neocortical areas. These results correspond to the notion of body odors representing significant social signals within the context of human mate choice, even with regard to communication of gender and sexual orientation. Moreover, preliminary results regarding behavioral effects of such body odors show that, if embedded as social context information, they affect basic levels of affiliative behavior.

#57 Between and within gender variations in the perception of human odour compounds

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Within human chemosensory communication, body odours and their components are discussed as carriers for information about gender and sexual orientation. Androstenone (5-alpha-androst-16-en-3-one) is one of the characteristical compounds of male body odour, binding to specific receptors located in the human olfactory epithelium. Here, we investigated whether the perception of androstenone depends on the sexual orientation.

Sensitivity to androstenone, odour-ratings of pleasantness, unpleasantness, intensity and familiarity as well as self-reported mood, regarding to valence, arousal and dominance were assessed in 13 homosexual and 14 heterosexual men (study 1, Lübke et al, 2009) as well as in 20 heterosexual women, 19 lesbian and 19 bisexual women (study 2). Isovaleric acid served as a control odour. A factorial design was used in study 1. In study 2, sexual orientation was correlated with olfactory sensitivity, odour-ratings and mood-self-reports.

Homosexual men showed a significant higher sensitivity to androstenone than heterosexual men (p=.031). Male groups did not differ in their sensitivity to isovaleric acid. There were no differences regarding odour-ratings or mood self-reports. More homosexually oriented women rated androstenone to be more intense (p=.044) and familiar (p=.028), and responded with a stronger arousal to the odour (p=.004). More heterosexually oriented women perceived androstenone to be less familiar (p=.033) and felt less aroused by it (p=.005).

Results indicate that sexual orientation significantly affects the perception of androstenone. Effects could result from differences in the endocrinological status of the perceivers or could be due to sensitization processes.

Lübke et al. (2009) Chem. Percept., 2, 154–160.

We thank R. Cole and R. Barthels for their contribution to data collection.

#58 The impact of sexual orientation on human olfaction

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An increasing volume of research has documented a biological basis to homosexuality in both males and females. Furthermore, a growing body of research has consistently demonstrated a degree of sex-atypicality among homosexual individuals on a wide variety of somatic, neuroanatomical and neuropsychological characteristics. However, the impact of an individual's sexual orientation on their olfactory function has, comparatively, received limited empirical investigation. This talk will briefly explore the extant research on this area focusing on the effects of sexual orientation on i) body odour production, ii) olfactory sensitivity, iii) the self-evaluated importance of olfaction and iv) the induction of olfactory sensitivity.

Oral Communications

Contributed Papers Oral Session 1: Invertebrates

#59 Insect perception of blends of host plant volatiles

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Plant volatiles are detected by the highly sensitive olfactory system of insects and are used for recognition of suitable plants as hosts and avoidance of unsuitable hosts. Detection depends on olfactory receptor neurones (ORNs) which are tuned to individual molecular structures (Bruce et al. 2005). However, perception of host odours is strongly influenced by volatile blend composition. There are emergent properties of blend perception because components of the host blend may not be recognised as host when perceived outside the context of that blend (Bruce & Pickett 2011). Different behavioural responses can occur to a whole blend compared to individual components (Webster et al. 2010). Often there is redundancy in the composition of blends recognised as host because certain compounds can be substituted by others. Fine spatio-temporal resolution of the synchronous firing of ORNs tuned to specific compounds enables insects to pick out relevant host odour cues against high background noise and with ephemeral exposure to the volatiles at varying concentrations. This task is challenging as they usually rely on ubiquitous plant volatiles and not those taxonomically characteristic of host plants. However, such an odour coding system has the advantage of providing flexibility; it allows for adaptation to changing environments by alterations in signal processing while maintaining the same peripheral olfactory receptors.

Bruce & Pickett (2011) Phytochem, 72, 1605–1611; Webster et al. (2010) Animal Behaviour, 79, 451–457; Bruce TJA et al. (2005) Trends Plant Sci, 10, 269–274.

#60 Adaptive gain control in the ON and OFF olfactory receptor neurons to fluctuating changes in odor concentration

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Sensory systems continually adjust their sensitivity to the current stimulus conditions. This flexibility relies on adaptation mechanisms that help match the range of input signals that a receptor neuron encounters to the range of its output. Adaptation of olfactory

receptor neurons (ORNs) has long been recognized to occur following strong odor concentrations, be they pulsed or constant. We focus here on the ability of the cockroach's ON and OFF ORNs to control dynamically the gain of their responses to slow and continuous changes in the concentration of fruit odor. The signals controlling gain are derived directly from the stimulus input or from a signal of the ORN itself. The ON and OFF ORNs generate excitatory responses that indicate either an increment or decrement in odor concentration, which enhances information about temporal concentration changes (Hinterwirth et al., 2004; Tichy et al., 2005; Burgstaller and Tichy, 2010). We found that during oscillating changes in the concentration of the odor of lemon oil the discharge rates of both types of ORNs are governed not only by the odor concentrations but also by the rate with which concentration changes. When odor concentration oscillates rapidly with brief periods, adaptation improves the gain for instantaneous concentration and reduces the gain for the rate of concentration change. Conversely, when odor concentration oscillates slowly with long periods, adaptation increases the gain for the rate of change at the expense of instantaneous concentration. We propose benefits of the adaptation-induced gain control in the ON and OFF ORNs.

Tichy H, Hinterwirth A, Gingl E (2005) Olfactory receptors on the cockroach antenna signal odour ON and odour OFF by excitation. European Journal of Neuroscience 22:3147–3160.

Burgstaller M, Tichy, H (2010) Functional asymmetries in cockroach ON and OFF olfactory receptor neurons. J. Neurophysiology 105:834–845

This work was supported by Austrian Science Fund Grant P 21777-B17.

#61 Molecular and cellular pathways of NaCl perception in *C. elegans*

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The nematode *C. elegans* is attracted to 0.1 to 200 mM NaCl and avoids higher NaCl concentrations. However, after prolonged exposure to NaCl the animals avoid all concentrations of NaCl, called gustatory plasticity. Attraction to NaCl is mainly mediated by one pair of sensory neurons, ASE, and involves the guanylate cyclases GCY-14 and GCY-22, the cyclic nucleotide gated (CNG) channel TAX-2/TAX-4 and calcineurin TAX-6/CNB-1. Osmotic avoidance is mediated by the ASH neurons and requires the Gα subunit ODR-3 and the TRPV channel subunit OSM-9. We have identified six new genes involved in attraction to NaCl, which function in two genetic pathways. One pathway involves *tax-2*, *tax-4*, *tax-6* and two MAPK genes. The second pathway consists of *tax-2,* the CNG channel subunit *cng-3*, *osm-9* and *odr-3*. We hypothesized that the response to NaCl is determined by a balance between attraction and avoidance. Using Ca^{2+} imaging we found that the ASE neurons of naïve animals respond to both low and high NaCl concentrations. The ASH neurons only respond to high NaCl concentrations. However, after prolonged exposure to NaCl, the ASE neurons are desensitized, while the ASH neurons are sensitized, which requires signals from ASE. Our results suggest that naïve *C. elegans* are attracted to NaCl, predominantly mediated by ASE, but that this attraction is overruled by osmotic avoidance, mediated by ASH. Pre-exposure to 100 mM NaCl in the absence of food, sensed by ASE and other neurons, changes this circuit, resulting in desensitization of attraction and sensitization of avoidance.

This study was supported by NWO/ALW.

Contributed Papers Oral session 2: Vertebrates

#62 Involvement of the G-protein Gαo in vomeronasal function and aggressive behavior in mice

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The rodent vomeronasal organ (VNO) is specialized in the perception of socially-relevant chemical stimuli and mediates the regulation of species-specific and interspecies social behaviors. The VNO is segregated into two zones containing subpopulations of vomeronasal sensory neurons (VSNs) which differ in their expression of chemosensory receptors and G-protein subunits [either Gnai2 (Gi2) or Gnao1 (Go)]. It is unclear what role the two segregated neural pathways play to control complex social behaviors such as aggression. We used conditional gene targeting to evaluate the role of the Gnao1 gene in a cell-type or organ-specific manner. We employed the Cre-loxP system to delete Go in those cells that express olfactory marker protein, which includes all vomeronasal sensory neurons (VSNs) of the basal layer of the VNO sensory epithelium. As a consequence of the deficiency, the number of VSNs that normally express Go decreased. VSN activation by some peptide and previously identified protein pheromones (Chamero et al, 2007) is drastically reduced in the Go mutants indicating that this G-protein is necessary for the detection of these chemosignals. Detection of other ligands specific for Gi2-expressing VSNs is not affected. Display of both maternal and male territorial aggression is severely diminished. However, unlike mice with genetically ablated VNO function, the Go mutants display a normal mating partner choice and sex behavior. These findings indicate that Go is required in the olfactory system to detect protein and peptide pheromones and is necessary to generate both maternal and male territorial aggressive behavior.

Chamero et al (2007) Nature 450:899–902.

This work was supported by the Deutsche Forschungsgemeinschaft (DFG), the VolkswagenStiftung, and the NIH Intramural Research Program of the NIH.

#63 Fgf signaling controls pharyngeal taste bud formation through miR-200 and Delta-Notch activity

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Taste buds, the taste sensory organs are conserved in vertebrates and composed of distinct cell types including taste receptor,

basal/presynaptic and support cells. Here we first characterize zebrafish taste bud development and show that compromised Fgf signaling in the larva results in taste bud reduction and disorganisation. We determine that Fgf activity is required within pharyngeal endoderm for formation of Calb2b+ cells and reveal *miR-200* and Delta-Notch signaling as key factors in this process. *miR-200* knocking down shows that *miR-200* activity is required for taste bud and in particular Calb2b+ cell formation. Compromised *delta* activity in *mib*-/- dramatically reduces the number of Calb2b+ cells and increases 5HT+ cells. Conversely, larvae with increased Notch activity and *ascl1a*-/- mutants, are devoid of 5HT+ cells but have maintained and increased Calb2b+ cells, respectively. These results show that Delta-Notch signaling is required for intact taste bud organ formation. Consistent with this, Notch activity restores Calb2b+ cell formation in pharyngeal endoderm with compromised Fgf signaling but not after *miR*-200 knockdown. Altogether this study provides genetic evidence supporting a novel model where Fgf regulates Delta-Notch signaling and subsequently *miR-200* activity to promote taste bud cell type differentiation.

This study was supported by FP6-ZF MODELS, ANR06-BLAN-0321, ANR-09-BLAN-077.

#64 Role of fila olfactoria in olfaction of *pseudapocryptes lanceolatus* (Bloch and Schneider) – a cytological demonstration

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Pseudapocryptes lanceolatus, a teleostean gobiid, possess unilamellar olfactory apparatus (i.e., associated with single olfactory lamella, accessory nasal sacs, olfactory nerve tract, olfactory bulb and brain) (Sarkar and De, 2011b). The olfactory lamella is externally lined by pseudostratified olfactory neuroepithelium (Sarkar and De, 2011a). Fila olfactoria or lamina propria is the ground element of the olfactory structure, present beneath the basal lamina. Present study focused on the microscopical details of the fila olfactoria and their probable role in olfaction. The olfactory lamella of P. lanceolatus was fixed in 2.5% glutaraldehyde in 0.1 M phosphate buffer (pH 7.2 – 7.4) for 1hour at 4°C and viewed under transmission electron microscope (TEM MORGAGNI-268D) operated at 40 kV. This very zone is characterized with aggregation of axons of different sensory receptor cells, collagen fibers, fibroblast cells, blood vessels with erythrocyte cells, blood cells, etc. The axon of different type of receptor cells are invading the basal lamina and aggregated at this region. This aggregation ultimately leads to the formation of olfactory nerve tract and leave the olfactory lamella to connect with the olfactory bulb of the brain. The axonal bundles are guarded by numerous collagen fibers and connective tissues. The fibroblast cells are frequently observed in this ground region. Fibroblast is a polarized cell and tapering at both ends. Chromatinized nucleus is elliptical in shape and centrally placed. Comparatively the euchromatin materials are greater than heterochromatins. The density of mitochondria is higher in perinuclear region. Polyribosomes and rough endoplasmic reticulums (rER) are also noted. The cis-trans axis of Golgi complex is very much prominent within the fibroblast cell and the vesicles are densely occupied by secretary substances. The primary lysosomes are well marked in different cytoplasmic region of fibroblast cells. It is reported the fibroblast cell may have different effects on the neuronal progenitor cells (Nakamura et al., 2002).

Nakamura et al., (2002) Eur Arch Otorhinolaryngol, 259, 166–169.
Sarkar and De (2011a) Int. J. Sci. Nat., 2(1), 1–6.
Sarkar and De (2011b) Int. J. Sci. Nat., 2(2), 186–191.

#65 Combinatorial encoding of mouse odour signatures by major urinary proteins (MUPs): evidence for modular principle of their decoding?

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Major urinary proteins (MUPs) of the house mouse belong to the highly diverse lipocalin superfamily (Flower, 1996). As a typical odorant-binding protein, MUP can bind a wide range of volatile pheromonally active ligands and thus can trigger several behavioural and physiological responses in recipients. Nowadays MUPs are considered as a key component in olfactory signature in *Mus musculus*: they can provide essential information about individuality and physiological state of donors (e.g., Roberts et al, 2010; Wyatt, 2010; Janotova, Stopka, 2011; Kwak et al, 2011) However, fine mechanisms of olfactory coding by MUPs and decoding MUP-odorant complexes remain to be elucidated (Novikov et al., 2011). Using electrophoresis in polyacrylamide gel (PAGE), we examined differential protein profiles in urine of genealogically unrelated CBA/LacY and C57BL/6JY laboratory mice according to genotype, sex, age, and physiological status. Quantitative evaluation of eight MUPs' fractions with different electrophoretic mobility (A-H) reveals that specific combination and proportion of individual fractions form two distinct protein subsets (modules). These subsets appear in both sexes very soon after weaning and resemble genotype-specific «bar codes» (Novikov et al, 2009). Taking into account that MUPs by themselves can efficiently activate vomeronasal neurons (Chamero et al, 2007, 2011) and that the expression patterns of vomeronasal receptors (V2Rs) are also combinatorial (Tirindelli et al., 2009) and sex-specific (He et al, 2008), we propose that molecular mechanisms of olfactory decoding the unique MUPs combination should be based on complementary chemical «dialogue» between individual subsets of MUPs and specific modules of V2Rs.

Flower (1996) Biochem. J., 318, 1–14; Roberts et al (2010) BMC Biol., 8, 75; Wyatt (2010) J. Comp. Physiol. A., 196, 685–700; Janotova, Stopka (2011) J. Chem. Ecol., 37, 647–656; Kwak et al (2011) Chem. Senses, 36, 443–452; Novikov et al (2011) Chem. Senses, 36, E31; Novikov et al (2009) Russian J. Develop. Biol., 40, 204–211; Chamero et al (2007) Nature, 450, 899–902; Chamero et al (2011) Proc. Natl. Acad. Sci. USA, doi: 10.1073/pnas. 1107770108; Tirindelli et al (2009) Physiol. Rev. 89, 921–956; He et al (2008) Science, 320, 535–656.

Supported by grants from Russian Foundation for Basic Research (projects 02-04-49273, 04-04-63050, 07-04-01762) and State Science and Technical Program «Priority Directions of Genetics».

#66 Afferent output in type II cells

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In taste cells of the type II, ATP-permeable channels mediate release of neurotransmitter ATP in response to taste and voltage stimulation. Evidence exists that pannexin1 (Panx1) hemichannels serve as a conduit of ATP release (Huang et al, 2007). The steep dependence of ATP release on membrane voltage suggests that afferent output in type II cells is driven by action potential (AP) rather than by gradual receptor potential (Romanov et al, 2007, 2008). Consistently, TTX, a VG Na^+-channel blocker, has been found to markedly impair ATP secretion from bitter responsive taste cells (Murata et al, 2010). At high depolarization elicited by AP, the electrochemical force driving ATP efflux is very small. It is therefore more effective, by analogy with exocytotic neurotransmitter release driven in certain synapses by tail Ca^{2+}-currents through VG Ca^{2+}-channels, to release the bulk of ATP during the re-polarization phase of AP, employing ATP-permeable channels deactivating relatively slow. To elucidate whether Panx1 hemichannels match the basic properties of ATP-permeable channels of type II cells, Panx1 was cloned from the CV papillae and heterologously expressed in HEK-293 cells. Transfected cells exhibited carbenoxolone-sensitive outwardly rectifying currents generally absent in control cells, thus indicating functional expression of Panx1 hemichannels. It was found that by biophysical and pharmacological properties, these recombinant channels were distinct from ATP-permeable channels of type II cells. Altogether, our data suggest that either Panx1 hemichannels do not largely mediate ATP release in type II cells or ATP-permeable channels are a heterooligomeric complex of Panx1 with other channel protein(s).

Huang et al (2007) Proc. Natl. Acad. Sci. USA, 104, 6436–6441; Romanov et al (2007) EMBO J. 26, 657–667; Romanov et al (2008) J.Gen. Physiol. 132, 731–744; Murata et al (2010) J. Neurophysiol. 104, 896–901.

This study was supported by Russian President Program for Young Scientists (MK-751.2010.4) and Russian Foundation for Basic Research (10-04-01230-a).

#67 Exploring the olfactory environment of premature newborns

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Numerous studies indicate that prematurity is associated with substantial developmental impairment in the newborn, but until now little attention has been directed to the olfactory environment to which premature newborns are exposed inside their incubators. A survey conducted in 99 neonatal units revealed that preterm infants are daily exposed to numerous dominant odours emanating from care and hygiene products (disinfectants used to clean materials, alcohol-based hand rubs, adhesive removers, soaps, etc). During their whole hospital stay, infants born at 32 and 28 weeks of gestation were estimated to be exposed to these odours an average of 2024 and 3448 times, respectively. As most of these odours were described as unpleasant and strongly pungent by an adult panel, a second study was conducted to quantify the health effects of exposure to these nosocomial odours. The physiological parameters of 25 premature infants aged 26-32 weeks of gestation were examined during 10 sec exposure to the 10 most frequently used odorous products. A decrease in respiratory rate associated with an increase in heart rate was the most frequent pattern of response. However, odorants with strong trigeminal component induced in mean a decrease of 30% in respiratory rate and 25% in heart rate. In some infants, episodes of bradycardia and blood oxygen desaturation were detected, indicating that preventive measures should be implemented to avoid unnecessary exposures in these micro-environments, while maintaining a high level of hygiene. Different strategies for neutralizing or masking these odours in the incubators are also actually under examination.

This study was supported by the CNRS and the French Ministry of Health (PHRC program).

Contributed Papers Oral session 3: Chemical Interactions

#68 Odorant – protein chemoreception observed through a computational microscope

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With the regular increase of both computational power and force-field quality, molecular modeling has become a force to reckon with. In many cases it can even be considered as a computational microscope, deciphering the dynamic interactions between molecular systems, varying in nature and size. With the use of such a tool and after a validation through comparison with biophysical data, we decipher the atomic-level nature of odorant / OBP and odorant / human-OR interactions. The dynamic behaviour of the entry/exit gates of these proteins are observed during the binding/unbinding processes. (Golebiowski et al, 2007; Charlier et al, 2011).

Golebiowski et al. Mechanistic events underlying odorant binding protein chemoreception PROTEINS: Structure, Function, and Bioinformatics (2007) 67, 448–458.

L. Charlier, et al. Chap. XX. Molecular Modeling of Olfactory Receptors. "*Methods in Molecular Biology. Olfactory Receptors*" Humana Press.Ed. (2011), in press.

#69 Large scale conducting polymer arrays emulating olfactory receptors

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We have great interest in understanding and emulating the sensing characteristics of biological olfactory systems that are characterised by large numbers of broadly tuned receptors that act as transducers for volatile chemicals. The high sensitivity and exquisite discriminatory capacity of biological olfactory systems is thought to be

derived from signal processing of the large numbers of sensory inputs that occurs within the olfactory bulb. In testing realistic computational models of the olfactory system large numbers of chemical sensor inputs are required. Synthetic data are not very satisfactory but large scale chemical sensor arrays that may serve as model inputs to an artificial olfactory system have not been available. Here we describe the development of a large scale array of chemical sensors based on organic conducting polymers. This consists of 16000 individual sensor elements with about 30 different types of materials that are deposited on electrodes with different geometries, allowing differing dynamic ranges of responses to be achieved. Using this system it is possible to start to test computational hypotheses appropriate to biological chemosensory systems and adapt them to the field of artificial olfaction.

#70 The redundancy reduction and data compression using cosine similarity and wavelet transformation at a very large chemical gas sensor array system

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Olfactory receptor neurons in the mammalian olfactory system transduce the odour information into electrical signals and project these into the olfactory bulb. In the biological system the huge redundancy and the massive convergence of the olfactory receptor neurons to the olfactory bulb are thought to enhance the sensitivity and selectivity of the system.

It was reported that the latest sensor technology allow an 65536 conductive polymer sensor array to be made with broad but overlapping selectivity to different families of chemicals emulating the characteristics found in biological chemoreception.

However, the supernumerary redundancy always accompanies great error and risk as well as an inordinate amount of computation time and local minima at signal processing, like neural networks. In this research, we used butterworth filter to smooth a data set and wavelet transformation to compress a data set as 1/64. After that, we applied the new method for redundancy reduction using cosine similarity method. By using the proposed method, data were reduced from 1000x4096 to 16x200. To prove the proposed method, we have done a comparative analysis between gas sensor data with a reduced redundancy and without the reduced redundancy and reached a satisfactory result.

#71 A bioelectronic sensor nanoplatform based on olfactory receptors and electrochemical impedance spectroscopy

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The European project BOND (Bioelectronic Olfactory Neuron Device) aims at using olfactory receptors (ORs) carried by nanoscale liposomes as functional elements of an electrochemical array, for specifically detecting target odorants. This project will integrate bio, micro/nano, and information technologies into an bioelectronic nanoplatform, benefiting the ORs high sensitivity to detect low odorant concentrations.

Here, the natural ORs relevant to monitor the presence/concentration of a target odorant are identified by single-cell RT-PCR on neurons exhibiting a calcic response to this odorant. The 3D structures of ORs relevant for this odorant can then be ranked relative to their binding efficiency. These ORs are then expressed in yeasts, and liposomes of nanometric scale, carrying the ORs on their lipid bilayer, are prepared from yeast membrane fraction. Surface Plasmon Resonance is used to monitor the ORs response to the target odorant.

The active part of the bioelectronic sensor nanoplatform is then developed, consisting of a silicon nanoelectrode structure with an electrochemical cell and a dedicated electronic chip for detecting very low level signals. To increase transducers surface and amplify the electrochemical transduction signal, gold nanoparticles are generated on the gold surface, using a chronoamperometry method to favor nucleation for electrochemical deposition. Nanosomes carrying ORs are immobilized using self-assembled monolayers techniques, and the biosensor properties monitored by Electrochemical Impedance Spectroscopy. Gold nanoparticles induce a surface area increase of about 2, measured by both EIS and AFM. Nanosomes immobilization leads to an increase in charge transfer resistance, an effect amplified in the presence of gold nanoparticles.

This study was supported by the European project BOND (228685-2) of the 7th PCRD. http://bondproject.org/.

#72 Smell the colour of the rainbow

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This paper explores a new 21st century body media multi-sensory 'rainbow', introducing computerized scent-output systems worn on the body for fashion, wellbeing and health applications. The rainbow merges technology and fashion with natural fragrances using the therapeutic power of essential oils to reflect the colours of the rainbow. The aim is to enhance mood and improve lifestyle, and will be placed alongside those in vogue such as alternative healing practices. It offers a fully personalized, controllable '*scent bubble*' experience which is intimate in nature and activated by the user alone from a collection of high-tech jewellery and clothing. Designed for psychological end benefits and as a luxury fashion item, this collection can be programmed to deliver a palette of fragrances, depending on emotion, mood, occasion and time of day. Building on earlier work on 'eScent®' (Tillotson et al, 2006), minute delivery mechanisms can produce a selection of aromatic molecules in controlled ways, responding to personal needs. If combined with biometric sensors that measure stress indicators, soothing scents

could be released whenever the stress levels exceed a certain threshold. Similarly, refreshing and revitalising scents can be used to fight fatigue and boost self-esteem. The ultimate goal is to embed electronic nose sensors within the structure of clothing to sniff stress and diseases. Using colour in conjunction with therapeutic fragrances, gives the wearer a visual aid to alleviating their physiological and emotional state. State of the art technology, 21st century design features and the healing powers of nature combine to produce a fundamentally simple concept of creating a positive personal space on this highly charged 21st century planet we all share.

Tillotson.J., Manz.A., Jenkins.G, (2006), Proc IET Seminar on MEMS & Actuators, 2006 April.

This study was supported by an Arts & Humanities Research Council (RCUK) Knowledge Transfer Fellowship in collaboration with Northumbria University and Philips Research.

Contributed Papers Oral session 4: Humans

#73 Altered savoury taste thresholds in overweight/obese children

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Obese women have significantly higher thresholds for monosodium glutamate (MSG), but not for other tastes (Pepino et al 2010). This study addressed the relationship between weight, and taste threshold and liking in children (<18 years).

Approval for the study was given by the relevant local Research Ethics Committees. Sixty nine children gave consent/assent;seven were subsequently excluded (age >18 on testing or incomplete data sets). Participants (n=62, age range 5-17 years) were weighed, and height measured and BMI centile-for-age calculated (range 4 - >97th centile). Taste thresholds for salt (NaCl), sweet (sucrose) and glutamate (MSG) were determined using a 2-alternative forced-choice staircase method. Liking for each taste was determined from a single presentation of 100mM NaCl, MSG and sucrose and rated using the ''Faces Scale'' (Andrews and Withey, 1976).

Salt and MSG discrimination thresholds were significantly correlated with BMI centile, whereas there was no association between sucrose threshold and weight. There were no differences in taste thresholds in boys, but overweight/obese girls (>85th centile) had significantly higher salt and MSG taste thresholds than normal weight girls. There were no significant differences in liking of different tastes in boys and girls of different weights.

These first data on savoury taste perception in children demonstrate that, as in adults, savoury taste thresholds are altered in females with high BMI compared to males and females of normal weight. Obesity, particularly in females, seems linked to a blunting of savoury taste.

Pepino et al (2010) Obesity, 18(5), 959-65; Andrews and Withy (1974) Social Indicators of Wellbeing: Americans' Perspective of Life Quality, Appendix A, p13.

This study was supported by a grant from the Charitable Trusts of University Hospital Bristol No. 300. We gratefully thank all the participants in the study, and the staff at COCO clinic Bristol Royal Infirmary and at St Mary Redcliffe and Temple School, Bristol.

#74 Nice to sniff you: olfactory sampling of conspecifics in humans

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Mounting evidence suggests that a portion of human behaviour, such as mate selection, is significantly influenced by odours. In that humans do not overtly sniff each other, one may ask how do humans obtain olfactory information on conspecifics. In Western society, the most common physical interaction with newly-met individuals is the handshake (Hall and Hall 1983). In an anecdotal incident we observed that following a handshake, humans often inadvertently sniff their own hands. To test the hypothesis that handshakes serve olfactory investigation, we filmed individuals for two minutes before and after they met a stranger (the experimenter), that either shook their hands or not (subjects were unaware of the filming). We then counted the instances where subjects brought the "hand-that-shook" to their nose, both before and after the meeting. An ANOVA revealed a significant interaction between the time (before/after meeting experimenter) and condition (handshake/no handshake) ($F(1,24)=6.1$, $p < 0.03$), reflecting a significantly increased rate of bringing the hand to the nose after a meeting with a handshake (0.64 ± 0.18 touches before vs. 1.28 ± 0.3 touches after, $t(24)=2.42$, $p < 0.03$)), but not after a meeting without a handshake (0.72 ± 0.14 touches before vs. 0.68 ± 0.19 touches after, $t(24)=0.19$, $p = 0.85$)). These results indicate that humans use handshakes to obtain olfactory information on conspecifics.

Hall, P.M. and Hall, D.A. (1983). The Handshake as Interaction. Semiotica 45, 249–264.

This study was supported by the Minerva Foundation.

#75 The relation of sexual orientation, gender nonconformity and olfactory abilities

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A vast body of research has shown that women tend to outperform men in various olfactory abilities. Further, homosexuals often exhibit gender atypical traits, as might well be the case with olfaction. We hypothesised that both in men and women sexual orientation would be correlated with olfactory scores and odour awareness but it would go in the opposite direction in women. The Sniffin' Sticks test was used to assess the olfactory threshold, discrimination and identification (TDI) of 40 homosexuals ($F=20$) and 40 heterosexuals ($F=20$) aged 20-35. Further, self-report Gender Nonconformity Scale (GN) and Odour Awareness Scale (OAS) were administered. A GLM analysis was performed, which yielded a significant sex difference and sex*sexual orientation interaction. Post-hoc tests revealed that this was due to a difference in the olfactory threshold, with heterosexual men being less sensitive than heterosexual women ($p=.017$); in the TDI, with heterosexual men scoring less than both homosexual men ($p=.009$) and heterosexual women ($p=.016$) and, finally, in the OAS score, with heterosexual men scoring less than heterosexual women ($p=.038$). Furthermore, it was found that the self-reported sexual orientation correlated with the identification and TDI scores in men, as did the GN score,

with homosexuals and those tending towards nonconformity outperforming the conformist ones. In women, the GN score correlated with the threshold and TDI scores, with the conformist ones outperforming those tending towards nonconformity. The results suggest differences between male and female homosexuality and that olfactory abilities correlate with gender nonconformity in both sexes.

This study was supported by the Grant Agency of the Czech Republic, Charles University Grant Agency and the Ministry of Education of the Czech Republic.

#76 A genome-wide association study indentifies new loci that underlie food preferences in Italian isolated populations

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Genetic variation in taste influences food preferences which play an important role in food choices. Only a few taste receptor genes have been implicated in food preferences (e.g., T2R family; T1R1; T1R3) but it is likely that many more genes play a direct or contributory role in this behaviour. We present the first genome wide scan identifying genes that underlie the food preferences of 3 isolated Italian populations from the Italian Network of Genetic Isolates (INGI). Subjects were 2,834 healthy, adults. They completed a food preference questionnaire on 55 common foods, rating their liking of each item on a 9 point scale ranging from "like extremely" to "dislike extremely". We identified 4 genome wide significant loci ($p<5x10-8$) for whole milk, hot tea, ice cream and chili pepper liking respectively, plus 29 suggestive loci ($p < 5x10-7$) for other foods. Tea liking was associated with LANCL1, a GPCR strongly expressed in mouse embryo olfactory epithelium. Chili pepper liking was associated with KANK4 which is expressed in the dorsal root ganglion. Whole milk liking was associated with CACNA2D3, a gene involved in nociception. Ice cream liking was associated with CADPS, a regulator of catecholamine release, and TAC1 which is involved in Substance P pain pathways. Our findings shed light on genes which have not been previously described for taste, but may have interesting functions related to food liking. A better understanding of the genetic basis of food preferences may provide insights into the causes and treatments of dietary diseases such as obesity.

#77 Genes for taste and food preferences in communities along the Silk Road

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Few studies have investigated the role of taste genes in general food preferences at the population level. We conducted a candidate gene study of food preferences in communities living along the Silk Road in the Caucasus region, Central Asia and Tajikistan. A total of 432 subjects completed a food preference questionnaire comprised of common foods specific to each culture. Subjects rated their liking of each item on a 5-point scale ranging from "like extremely" to "dislike extremely". Liking rating was used as a quantitative variable in the statistical analyses. We selected 88 SNPs on 26 taste-related genes for genotyping, and we used linear mixed model regression analysis to test for associations. Alpha was set at $p\leq0.00238$. Liking of tea was associated with the PCLB2 gene ($p=1.5x10-03$) which is expressed in type II taste buds cells and in olfactory epithelium, and is involved in the response to caffeine. Liking of vodka ($p=4.6x10-4$) and white wine ($p=6.2x10-4$) was associated with the sweet receptor T1R2, supporting the idea that sweet taste contributes to variation in liking for alcohol. Preferences for lamb, peas, sheep meat, cheese, fava beans, and watermelon were associated with different variants of ITPR3, a gene expressed in the taste and olfactory systems. Finally, liking for radish was associated with TRPV1 ($p=1.07x10-05$) which has been implicated in oral irritation from isothiocynates, a principle compound in radishes. These findings suggest that a candidate gene approach is an effective tool for gaining insights into food preference variations in the general population.

#78 Olfactory white: mixtures containing many odorants converge to a common olfactory percept

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In vision, mixtures that combine all visible wavelengths, each at equal intensity, are perceived as *white*. In audition, sounds that combine all heard frequencies, each at equal amplitude, are perceived as a hum termed *white noise*. Here we found a similar perceptual phenomenon in olfaction. We conducted pairwise similarity rating experiments for 191 different odorant mixtures in 150 subjects. We found that as we added equal-intensity components that spanned olfactory space to each of two mixtures, the mixtures became more similar to each other, despite not sharing a single component in common ($Z = 2.36$, $p < 0.02$). Remarkably, from ~30 components, all mixtures began to smell similar, obtaining a quality we called *olfactory white*. Subjects acquainted with this white odor later preferentially identified other novel odorant mixtures as white, as long as they were made of ~30 equal-intensity well-spanned components or more. Such early perceptual morphing poses computational boundaries for the olfactory system, and like auditory *white noise*, may find its way into everyday life applications.

Posters

#P1. Stimulation of human olfactory system with fMRI: Group analysis in healthy subjects

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The human olfactory system *in vivo* is difficult to study with fMRI for the presence of magnetic susceptibility changes that cause signal losses and artefacts. The aim of this work is to develop a fMRI technique to produce robust and reproducible results in group analysis. It was evaluated different pattern of activation for the menthol (M) and heliotropyl acetone (H). A MR-compatible olfactometer was developed. 22 normal subjects (11 males, 11 females, 30.73±5.10 ys) underwent to fMRI experiments. Different temporal paradigms for the stimulation timing were tested: a regular and a randomized block design. The fMRI data set was analyzed by AFNI software. Multiple linear regression was used to analyze the data, by means of tent shaped basis functions. For the group analysis, a random subject analysis was performed using a t test on average signal changes, estimated at subject level. The main areas of the olfactory system were revealed both with M and H. Comparing the areas involved in the perception of M and H, employing the regular block design paradigm, we found that left cingulum, right fronto-lateral area and right orbito-fronto lateral were commonly activated; applying the randomized paradigm common areas were the left cingulum, the inferior temporal circonvolution of both the sides, both insula, right parietal supramarginal areas, right superior frontal gyrus and right pre frontal area.

This method could be effective for physiological studies and for clinical trials in altered sense of smell where group analysis is important.

#P2. Olfactory functioning in individuals high in psychometric schizotypy

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Olfactory deficits have been well documented in patients with schizophrenia and their first-degree relatives, however less is known about olfaction in individuals possessing sub-clinical manifestations of schizophrenia. The aim of the present study was to examine odor perception in individuals with psychometric schizotypia. Potential participants were invited to complete an online questionnaire comprising the Schizotypal Personality Questionnaire (SPQ) and a control scale (the Minnesota Multiphasic Personality Inventory-2 Lie Scale, MMPI-2 L-scale). Individuals who scored below 9 on the MMPI-2 L-scale and scored 41 and above (schizotypia cutoff) or 12 and below (control cutoff) on the SPQ were invited to complete an olfactory testing session. Of the invited individuals, 24 participants with psychometric schizotypia and 35 controls met the eligibility criteria (including the negative personal and family history of psychiatric disorders) and agreed to participate in the olfactory session. Participants completed the Sniffin' Sticks tests (n-butanol threshold, odor discrimination, odor identification), rated odors (citral, cinnamaldehyde, grapefruit oil, bergamot oil, hexylamine, butyric acid, guiaicol, pyridine) for pleasantness, intensity, and familiarity on 10-cm visual analogue scales, and completed the Beck Depression Inventory (BDI) and Beck Anxiety Inventory (BAI). Analyses showed that there were no significant differences between schizotypy and control group for the Sniffin' Sticks tests of n-butanol threshold, odor discrimination, or odor identification. Multivariate analyses of variance for the odor ratings showed that the schizotypy group rated hexylamine significantly more intense than the control group did (p=0.01). In summary, individuals with psychometric schizotypia display little to no deficits in olfactory function.

Acknowledgments: This study was supported by an NSERC Discovery Grant for J.D. and an NSERC Undergraduate Student Research Award for C.A.G.

#P3. Human olfactory dysfunction after traumatic brain injury

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Head injury is one of the major etiology of olfactory dysfunction in humans. The olfactory sequels would be permanent after a few months of evolution. Victims and their healthcare surroundings need an objective assessment of the olfactory function and its future development, if any.

We present here the results of the olfactory investigations, Magnetic Resonnance Imaging (MRI) and Olfactory Event Related Potentials (OERPs) for 34 consecutive patients victims of head injury investigated in our ENT departement (23 women and 11 men). The average post-injury follow up was 3 years ±2 (S.D.) The patients' group has been compared with 25 controls (14 women and 11 men).

We examined early components of OERPs following olfactory stimulations and Contingent Negative Variation (CNV) induced by a delayed motor task, presumed to reflect cognitive treatment of the warning (olfactory) stimulus and preparation to a motor task.

There are no OERPs in case of complete necrosis of the olfactory bulbs (OB).

The wave P2 asserts the specific stimulation of the olfactory pathway (OP). If present, it appears at Fp2 and Cz.

The CNV wave expresses the conscient perception of the olfactory stimulation. In contrast, we found in some patients a specific olfactory activation (P2 wave) without any awareness (lack of CNV wave).

In forensic examination, a patient complaining of anosmia after head trauma must undergo MRI and OERPs recordings. If there is evidence of P2 and CNV waves, one can assert the functionality but not the accuracy of the intracranial OP.

Anatomical evidence of OB and evidence of P2 and CNV waves, allow anticipations upon the olfactory rehabilitation.

#P4. Analysis of distinctive features of olfactory dysfunction caused by smoking and investigation of method of adjustment

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Prior studies have been ambivalent as to the effect of smoking on olfaction, however research suggests that olfactory dysfunction caused by cigarettes solely derives from chemicals present within tobacco smoke (Brämerson et al. 2004; Katotomichelakis et al. 2007). The aim of this study was to identify distinctive features of olfactory disturbance (if any) caused by cigarette smoking.

Forty participants (20 smokers and 20 non-smokers) were assessed by their olfactory thresholds for two odorants, n-butanol and phenyl ethyl alcohol (PEA), using a forced choice ascending staircase method. Both these odorants have been demonstrated to have similar olfactory thresholds (Croy et al. 2009). However, PEA is present in tobacco and tobacco smoke, whilst n-butanol is absent.

n-butanol threshold scores were compared between smokers and non-smokers using ANOVA. ANCOVA was then conducted to control varying n-butanol scores when comparing groups for PEA thresholds. This was implemented to determine whether PEA is subject to reduced threshold scores for smokers due to habituation. These results were compared with data obtained from an olfactometer using a participant-adjusted scale. The method of adjustment has not yet been implemented as an olfactory measurement technique. This new method may present a more efficient method of olfactory testing.

Preliminary results indicate that smokers do not have reduced olfactory threshold acuity beyond that of n-butanol, although both odorants required stronger concentrations to be detected by smokers. The method of adjustment is being found to correlate strongly with sniff bottle threshold scores indicating that it may be an alternative for olfactory tests.

Brämerson, A., Johansson, L., Ek, L., Nordin, S. and Bende, M. (2004), Prevalence of Olfactory Dysfunction: The Skövde Population-Based Study. The Laryngoscope, 114: 733–737.

Katotomichelakis, M., Balatsouras, D., Tripsianis, G., Davris S., Maroudias, N., Danieldes, V. and Simopoulos, C. (2007) The effect of smoking on the olfactory function. Rhinology, 45: 273–280.

Croy, I., Lange, L., Krone, F., Negoias, S., Seo, H-S. and Hummel, T. (2009), Comparison between Odor Thresholds for Phenyl Ethyl Alcohol and Butanol. Chemical Senses, 34(6): 523–527.

The authors would like to thank David Laing for his suggestions and assistance.

#P5. Effect from the fragrance of fabric softener on towels

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We studied the effects of various kinds of fragrance from fabric softener and its effect on towels. Preceding studies showed that towels presented with fragrance felt softer than non-fragrance towels. To evaluate the relation of odor and touch, excluding the visual information on the color or externals, etc., the examination method using a Head Mounted Display was chosen. Participants evaluated the texture on a visual analog scale, while touching the towel which create a constant softness in the draft chamber with which the fragrance was filled, along with the instruction displayed in Head Mounted Display. We prepared 9 kinds of fabric softeners, and classified them into 2 fragrance types; fresh green type and sweet floral type. As a result of the evaluation, participants felt the softness of the towel while smelling the sweet floral type rather than fragrance of the fresh type. The results from the softness evaluations of the towel were correlative with preference ratings of fragrance ($p<0.01$), but not with strength ratings of fragrance ($p>0.29$). This tendency was more obvious in the case of the sweet floral type fragrance. Theseresults suggest that there was cross-modal interaction between fragrance and touch, but this phenomenon depended on the fragrance type. We experience this phenomenon in our daily lives. For example, consumer's evaluation in home use test also showed that fragrance of fabric softener emphasized a sense of touch in accordance with this study.

#P6. Calcium Responses to Sulfated Steroids in Neurons of the Mouse Vomeronasal Organ

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In most mammals, chemosensation involves the activation of at least two major sensory systems: the main and the accessory (vomeronasal) olfactory systems. The vomeronasal organ is located at the base of the nasal cavity and plays important roles in many behaviors.

Recent studies have identified sulfated steroids (products of steroidal hormones catabolism) as one of major activator of neurons of the vomeronasal organ, and are therefore a useful tool to investigate the coding of stimuli in these neurons. We have measured the intracellular calcium concentration changes induced by the application of sulfated steroids to neurons isolated from the vomeronasal organ of female mice. We found that a stimulus made by a mix of ten sulfated steroids from the androgen, estrogen, pregnanolone, and glucocorticoid families induced a calcium response in a higher percentage of neurons (71%) compared to urine (28%). Moreover, 37% of the neurons responded to a mix composed of three glucocorticoid-derived compounds, and 26% of the neurons responded to a mix composed of three pregnanolone-derived compounds. Neuron responses were highly specific, with no neurons that responded to both mixes. We found that some neurons responded to more than one individual component of the glucocorticoid-derived mix indicating that some neurons of the vomeronasal organ are broadly tuned although they still displayed strong specificity, remaining unresponsive to high concentrations of ineffective compounds.

We thank Michele Dibattista and all members of the laboratory for discussions. We acknowledge the technical assistance of Beatrice Pastore.

#P7. Does the view of your nose help your smell?

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Vision of one's own body part can improve tactile sensitivity on that body part (Serino et al, 2009). Here, we verified whether seeing one's own nose could facilitate odour detection, and eventually whether this effect could affect the olfactory and the trigeminal systems differently. Therefore, we presented 21 people (12 females) with an odour detection task by presenting through an olfactometer a series of stimuli (banana as odorant, mint as trigeminal stimulus, and clean air as control) while viewing on a PC monitor different pictures: Their own nose, a body-unrelated object (a kitchen hood), and a black square as control. On each trial, participants were instructed to try and detect the presence of odours as fast and accurately as possible. The results revealed that detection of the odorant was less accurate (M=85%) than both trigeminal stimulus and clean air (for both, M=97%). Overall, responses to clean air were significantly longer (M=1954 ms) than to trigeminal and odorant stimuli (M=1519 ms and M=1676 ms, respectively; cf. Jacob & Wang 2006). Interestingly, the interaction between odours and pictures was significant. As a matter of fact, responses to trigeminal stimuli were faster than those to odorant trials in both the control (M=1553 ms and M=1681 ms, respectively) and body-unrelated condition (M=1448 ms and M=1708 ms, respectively), while there was no difference in the body-related condition. This last result might be due to a possible distracting effect exerted by the nose picture which could have somewhat slowed down the responses to trigeminal trials.

Jacob & Wang (2006) Physiol. Behav., 87, 500–505; Serino et al (2009) Cortex, 45, 602–609.

This study was supported by PAT (AP 2009–2011).

#P8. Improved olfaction under hypnosis

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Human olfaction is characterized by high acuity yet low awareness to olfactory stimuli. This state has merited the hypothesis that the human olfactory system is under significant cortical inhibition. We further hypothesized that reducing cortical inhibition may improve olfactory performance. To test this, we set out to measure olfactory performance in disinhibited states. One method to generate disinhibition is by hypnosis. We proceeded to test olfactory performance in normal waking state and during a hypnotic state of reduced inhibition. To test olfactory performance we used an ascending-staircase three-alternative forced choice detection threshold test with the odorant PEA. We applied seven reversals after three consecutive correct detections, and took the mean concentration of the last 4. To date, we have tested 11 subjects (5 females, mean age = 26.5). Overall, 9 subjects had better olfactory performance under hypnosis compared to wake and two were better in the waking state compared to hypnosis. This overall improvement under hypnosis (binomial, $p < 0.04$) reflected a median 10% ± 9 improvement in the mean of the last 4 dilution steps (median 14% ± 5 in the 9 subjects that improved). These results suggest that olfactory performance can improve under hypnosis. Hypnosis, however, may have influence through a host of processes unrelated to disinhibition, e.g., increased concentration/attention. Thus, validating our hypothesis on disinhibition depends on additional tests, such as tests with disinhibiting pharmacological agents.

#P9. A pluridisciplinary study on a Japanese traditional olfactory art: Koh-do

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The aim of our KODO project (supported by the French National Research Agency, ANR) is to better understand the conditions of emergence of an Olfactory Art. The Koh-do ceremony, a Japanese traditional olfactory art, is taken as a model to study. The master of ceremony presents his art and knowledge to help the participants to open their mind to the odours. The participants appreciate the master's art and knowledge, and they focus on ''listening'' to subtle differences in fragrances of rare natural woods, called jinko (*Aquilaria agallocha*). The aim of the Koh-do ceremony is to find their own way (Do) with beautiful odours (Koh). In a ceremony, the odours are often associated with a poem describing a season, a scenery, evoking an emotion. The association of the poem with the odours helps the participants to fully appreciate the odors nuances.

Our project comprises 3 axes: 1) A philosophical, historical and sociological approach of Koh-do (conditions of emergence of such an art; Chantal Jaquet). 2) A neurophysiologial approach of the brain activity underlying the practice of Kho-do (Akiko Ishii-Forêt, Didier Trotier). 3) Olfaction and Creativity: integration of Olfaction in artistic practices, e.g. "scented" theater, and management of olfactory creativity (Sophie Domisseck, Roland Salesse).

Some more details of the Koh-do ceremony and our project will be presented.

Takagi SF (1989) The art of Smell, in Human Olfaction, University of Tokyo Pres, Tokyo.

Kiyoko Morita (2006), Book of incense -enjoying the traditional art of Japanese scents-, Kodansya International Ltd., Tokyo.

This study is supported by The French National Research Agency.

#P10. EEG and Koh-do practice

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One goal of the practice of Koh-do* is to help participants to open their mind to odours. During the Koh-do ceremony the subject focuses on the fugacity of the olfactory perceptions, elicited by small pieces of various fragrant woods, in order to detect and memorize subtle smell differences. Often the odours are associated with elements of a poem, adding a context to the odours. The synergy between the odours and the realm of a poem helps the participants to appreciate the nuance of the fragrance.

Using experimental conditions similar to a kumiko game, we record the EEG activity with 32 active scalp electrodes (BioSemi) as well as the subject's nasal airflow with a pressure sensor. The subject first memorizes one odour as a reference. Then, five odours are presented in a random order at an time interval of 60s : the reference odour, another odour presented three times and another one

presented only once. Only 3 inspirations are allowed for each sample. At the end the subject must write the order of presentation of the three odours.

Our aim is to find some electrophysiological cues underlying the Koh-do practice and to examine the effect of learning. Power spectra and time-frequency analysis, performed with EEGLAB and ERPWAVELAB softwares, as well as the independent components extracted by Independent Component Analysis, are examined in terms of their scalp topography.

* a Japanese traditional olfactory art.

Takagi SF (1989) The art of Smell, in Human Olfaction, University of Tokyo Pres, Tokyo.

Kiyoko Morita (2006), Book of incense -enjoying the traditional art of Japanese scents-, Kodansya International Ltd., Tokyo.

This study was supported by The French National Research Agency.

#P11. Temporal processing of chemosensory stimuli

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It is well known that in depressive patients, emotionally negative stimuli elicit differential or early processing of Event Related Potentials (ERP). However, the effects of odor pleasantness on the chemosensory event related potential (CSERP) is not eminent (Pause et al 2003). Conversely, within that past two decades, few studies have shown a correlation between odor valance and the differential processing of components of the ERP (Kobal et al 1992; Lundstrom et al 2006). In a recent study, Croy et al. (2010) showed that in Post Traumatic Stress Disorder (PTSD), the N1 latency for unpleasant irritation was accelerated in comparison to pleasant smells. By utilizing air-dilution olfactometry we examined whether the accelerated N1 latency is based upon an instantaneous processing event that occurs upon the initial stimulus delivery or whether the phenomenon is due to the effect of priming and sensitization processes. Forty-two healthy volunteers participated in 2 sessions where CSERP were recorded in response to olfactory stimulation with 3 hedonically different smells (H_2S unpleasant rotten egg smell, Phenyl ethyl alcohol; PEA rose-like neutral odor and Peach pleasant fruity smell) of analogous intensities. The results for this study are currently analyzed and will be discussed. With little knowledge about the temporal processing of different chemosensory stimuli, we hope to deduce whether chemosensory priming plays a role in expediting the processing of unpleasant stimuli.

Croy et al (2010) International J of Psychophysiology, 75, 326-331; Kobal et al (1992) Chemical Senses, 17, 233–244; Lundström et al (2006) Chemical senses, 31, 705–711; Pause et al (2003). J of Psychophysiology, 40, 209–225.

#P12. The scent of human aggression decreases trust in men and women

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In rodents, aggression promoting chemosignals exert a priming effect, allowing anticipation of an impending attack and furthermore inducing aggressiveness or defensive behaviour in the perceiving animal. Previously, we could demonstrate that chemosensory signals of competition guide attention and orientation processes in humans. Here, we focus on the behavioural change affected by the smelling of human odor collected during an aggression induction (Cherek, 1981). We investigated, whether smelling human chemosignals of aggression would influence the expression of trust as a mechanism of social cooperation. The display of trust can be described as an expression of intention to affiliate with another party and requires the acceptance of vulnerability and the expectation not to be harmed or exposed to risk by the action of another.

We employed a modified version of the Trust Game (Berg et al., 1995) and presented 30 female and 30 male participants with 3 types of chemosensory stimuli (aggression odor, sport odor or plain air). We had all participants rate all stimuli according to intensity, unpleasantness and arousal before the start of the game. We found only aggression odor to significantly decrease the expression of trust ($p < .01$). Participants rated aggression odor as more intense and unpleasant than sport odor or air and felt most aroused when smelling the sweat sampled during the aggression condition. Although sport odor was perceived to be more intense and unpleasant than air, no significant behavioural change occurred under influence of sport odor ($p > .05$). There were no gender effects on neither behaviour nor odor rating.

Cherek, D.R. (1981) Psychopharmacology, 75, 339–345

Berg et al. (1995) Games Econ. Behav., 19, 122–142.

#P13. Unconscious odour perception: a case study of blind smell

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It is well documented that unconsciously detected chemicals may affect human behavior (Kirk-Smith et al, 1983; Stern and McClintock, 1998; Zucco et al, 2009) and brain activity (Lorig et al, 1990; Sobel et al, 1999). No studies, however, have investigated blind smell, namely, the olfactory counterpart of blindsight (Weiskrantz et al, 1974). In the present report, free and cued olfactory identification of suprathreshold odorants and taste and flavor identification abilities were examined in a patient (MB) who underwent left fronto-basal surgery for a meningioma at the level of middle-left ethmoid. MR imaging revealed encephalomalacia in the left gyrus rectus, with left olfactory bulb ablation and damage to the right bulb, subcortical abnormality on the left near the orbital cortex, and damage to a small section of the right gyrus rectus. On free identification MB, while denying any capacity to smell the stimuli, still correctly identified some odorants and described their qualities including odour-induced tastes (Stevenson et al., 1998). In addition, MB performed above-chance on cued odour identification. No taste impairments were observed. An interpretation of the present outcomes relying on the brain regions that might be involved in unconscious odour perception is tentatively provided.

Kirk-Smith et al (1983) Biol. Psych, 17, 221–231; Lorig et al (1990) Bull. Psychonom. Soc, 28, 405–408; Sobel et al (1999) Brain, 12, 209–217; Stern and McClintock (1998) Nature, 392, 177–179; Stevenson

et al (1998), Learn. Motiv, 29, 113–132; Weiskrantz et al (1974) Brain, 97, 709–728; Zucco et al (2009) Learn. Motiv, 40, 364–375.

#P14. Slow breathing and emotions associated with odor-induced autobiographical memories

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An important feature of olfactory perception is its dependence on respiratory activity. By inspiring, olfactory information ascends directly to olfactory-related limbic structures. Therefore, every breath including odor molecules activates these limbic areas associated with emotional experience and memory retrieval with respiratory changes. We tested that if odors associated with autobiographical memories can trigger pleasant emotional experiences, whether respiration changes during stimulation with these odors.

During presentations of odor related to autobiographical memories and control odors, we measured minute ventilation, tidal volume, respiratory frequency, O_2 consumption and end tidal CO_2 concentration. The present study provides evidence that autobiographical memory retrieval was associated with increasing tidal volume and decreasing respiratory frequency more than those during presentation of control odors. Subjective feelings such as emotional arousal during retrieval of the memory, arousal level of the memory itself, or pleasantness and familiarity toward the odor evoked by autobiographical memory were more specific compared with those related to control odors. This phenomenon was not caused by metabolic demand, confirmed by unchanged O_2 consumption, but was instead caused by inputs from higher brain centers. In addition, high trait anxiety subjects responded with a stronger feeling of being taken back in time and had high arousal levels with tidal volume increases. We will present regarding how deep and slow breathing is related to pleasantness and comfortableness of an autobiographical memory.

#P15. Identification of aptamers that bind to human sweet taste receptor (T1R2/T1R3)

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It has been shown that sweet taste is transduced by the Class III G Protein–Coupled Receptors (GPCRs) T1R2 and T1R3, which show long N-termini that form a globular extracellular ligand-binding domain. These receptors are expressed in the taste cells (epithelial cells that constitute the taste buds in taste papillae) that respond to sweet tastants. When T1R2 and T1R3 are coexpressed in heterologous cells, they respond, as heteromers, to a series of sugars, some D-amino acids, artificial sweeteners and sweet proteins. We used the combinatorial oligonucleotide library screening approach denominated *Systematic Evolution of Ligands by Exponential Enrichment* (SELEX) to isolate nuclease-resistant RNA aptamers that bind to the human sweet taste receptor with high affinity. Following a four round enrichment of the previous random RNA pool, the RNA was reverse-transcribed for DNA sequencing and verification of the efficacy of the technique. We next performed five more rounds and two negative counterselection cycles (to eliminate RNA molecules that bind nonspecifically to the nitrocellulose membrane and to proteins other than the target). Aptamers with consensus sequences were obtained. We plan now to perform intracellular calcium flux functional assays with heterologous cells expressing T1R2 and T1R3 in order to evaluate the biological activities of these aptamers.

Ulrich and Wrenger (2009) Cytometry A., 75(9):727-33; Ulrich, Martins and Pesquero (2005) Curr Protoc Cytom, Chapter 7: Unit 7.28; Malnic and Gonzalez-Kristeller (2009) Ann N Y Acad Sci, 1170:150-2; Malnic and Kerr (2009) Chem Senses, 34(1):25-6.

This study is supported by FAPESP and CNPq.

#P16. Large conductance channels recorded in mouse fungiform taste bud cells

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In taste buds, activation of type 2 (receptor) cells leads to the release of ATP through large conductance hemichannels, namely connexin (Romanov et al, 2007,2008) or pannexin (Huang et al, 2007). ATP then activates purinergic receptors on presynaptic (type 3) cells or nerve fibers.

Cell-attached recordings of fungiform mouse taste bud cells, in an isolated lingual epithelium maintained in Ringer, revealed the existence of functional channels with large conductance (>300pS for 4 cells). In most cases the activity appeared only after a few minutes of recording. Usually this activity consisted in rapid transitions between a closed state, or a low conducting state (10-40pS), to a large conducting level, as well as smaller transitions between numerous subconductance levels. The reversal potential indicated a non specific conductance. The probability of opening was, in our recording conditions, little dependent on the membrane potential. Our hypothesis is that the activity mainly depended on an intracellular cofactor. The fact that this activity appeared only after several minutes of recording also suggested the possible existence of an external inhibitory cofactor washed out in the pipette solution (CsCl 120mM, HEPES 10mM pH7.2). The existence of subconductance states suggests that the size of the pore might influence the nature of the ions passing through.

Huang, Y.J., et al., *The role of pannexin 1 hemichannels in ATP release and cell-cell communication in mouse taste buds.* Proc.Natl.Acad.Sci U.S.A, 2007. **104**(15): p. 6436–6441.

Romanov, R.A., et al., *Afferent neurotransmission mediated by hemichannels in mammalian taste cells.* EMBO J., 2007. **26**(3): p. 657–667.

Romanov, R.A., et al., *Voltage dependence of ATP secretion in mammalian taste cells.* J.Gen.Physiol, 2008. **132**(6): p. 731–744.

#P17. Expression of kainate glutamate receptors in isolated single cells from rat taste buds

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Glutamate-induced cobalt uptake reveals that non-NMDA glutamate receptors (GluRs) are present in rat taste bud cells. Previous studies involving glutamate induced cobalt staining suggest this uptake mainly occurs via kainate type GluRs. It is not known which of the 4 types of taste bud cells express subunits of kainate GluR. Circumvallate and foliate papillae of Sprague-Dawley rats (45~60 days old) were used to search for the mRNAs of subunits of non-NMDA GluRs using RT-PCR with specific primers for GluR1-7, KA1 and KA2. We also performed RT-PCR for GluR5, KA1, PLCbeta2, and NCAM/SNAP 25 in isolated single cells from taste buds. Taste epithelium, including circumvallate or foliate papilla, express mRNAs of GluR5 and KA1. However, non-taste tongue epithelium expresses no subunits of non-NMDA GluRs.

Isolated single cell RT-PCR reveals that the mRNAs of GluR5 and KA1 are preferentially expressed in Type II and Type III cells over Type I cells.

#P18. Labellar taste responses to host-fruits in the medfly *Ceratitis capitata*

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The medfly (*Ceratitis capitata* Wied.) is a widespread pest for horticulture, mainly targeting fruits of pomaceous and citrus cultivars. Scanty data are available on taste chemoreception, about tarsal and labellar responses to salts and sugars [1, 2]. HRSEM and TEM analysis has shown 6 pairs of ''Largest'' trichoid labellar chemosensilla, each containing 4 different neurons [4].We studied the responses of the medfly labellar taste chemosensilla to various parts (skin and pulp cold-pressed extracts and whole fruit washing) of some host-fruits (orange, apple, prickly pear and lemon) and related compounds (malic and citric acid) also in relation to sex, by means of extracellular single sensillum recordings [3]. 4 different neurons were found to respond to the test stimuli, on the basis of their spike amplitudes and shapes. Malic acid (an apple compound), at the two lowest concentrations, elicited relevant responses from 3 out of 4 cells, which instead responded weakly at the highest concentration, probably due to a pH effect which, in the case of citric acid (citrus fruit), produced an inverse dose-response relationship at all concentrations.

Spike responses to skin and pulp cold-pressed extracts of apple, prickly pear and orange, were multi-unit and high frequency, while whole fruit washings evoked weaker responses. Finally, multi-unit responses to cold-press extracts of prickly pear and apple were highest in frequency, but low to cold-pressed lemon extract. These results provide additional information about the response profiles to host-fruits in the 2 sexes of the medfly in relation to feeding and egg-laying.

1) Angioy et al., (1978). Boll. Soc. Ital. Biol. Sper. 21:2105–2107. 2) Gothilf et al., (1971). J. Insect Physiol. 17:1371–1384. 3) Hodgson et al., (1955). Science 122:417– 418. 4) Masala et al., (2010). ECRO Congress Avignon, 172 (P119).

#P19. Amygdala-perirhinal cortex interaction involved in the attenuation of taste neophobia

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Taste neophobia is defined as a reduced consumption of a novel taste solution relative to later exposures while it becomes familiar. If ingestion is not followed by negative consequences the neophobic response undergoes attenuation, thus evidencing the formation of a safe taste memory. Previous lesion data indicated that the habituation of taste neophobia requires the integrity of both Amygdala basolateral (Ambl) and Perirhinal cortex (PER). However, it is often difficult to behaviorally dissociate lesion-induced impairments on taste neophobia and its attenuation. Moreover a potentially relevant interaction between AMbl and PER has not been previously investigated. Thus, we have assessed the effect of bilateral neurotoxic lesions of AMbl on PER activity induced by both processes. Wistar male rats with NMDA lesions of the AMbl (AMbl-L; n=10) and sham-operated (SHAM; n=10) received two consecutive exposures (15 m each) to a 3% cider vinegar solution. Fos-like immunoreactivity was examined as a marker of neuronal activity in PER. As expected AMbl-L group showed no evidence of neophobia attenuation. A similar number of PER c-fos positive neurons were found in SHAM and AMbl-L groups exposed to the novel taste solution. However, AMbl-L group exhibited a lower number of c-fos stained neurons than SHAM groups when exposed to the familiar taste solution. This supports a role of PER in safe taste memory that depends on the integrity of AMbl. Therefore, these results suggest a general role of PER in recognition memory, including safe taste recognition memory.

Grants PSI2008-03933 (MICINN. Spain) and HUM 02763 (Junta de Andalucía. Spain) both supported by FEDER funding.

#P20. The role of Gα14 in taste signal transduction

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G protein-coupled receptors serve as taste receptors, and there are regional differences in expression patterns of G proteins. The sweet-receptive taste cells of the posterior tongue express Gα14, while those in anterior taste buds express Gα gustducin, a key molecule for sweet, umami and bitter taste signal transduction. Here, to investigate whether Gα14 mediates sweet taste of the posterior tongue, we measured chorda tympani and glossopharyngeal nerve responses to various taste stimuli in Gα14 knock-out (KO) mice in comparison with wild type (WT) mice. We found that in both gustatory nerves, responses to sweet, acids, salts, bitter and umami taste stimuli did not differ significantly between Gα14 KO and WT mice. Therefore, our results do not support a role for Gα14 in sensing of sweet or other taste qualities. Further studies are needed to elucidate the function of Gα14 in taste bud cells.

Shindo et al (2008) BBRC, 376, 504–508; Marco et al (2008) BMC Neurosci. 9, 110

#P21. Different populations of bitter taste receptor cells in mice

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Many bitter compounds are toxic and avoided. On the other hand some bitter compounds elicit beneficial health effects and contribute to the enjoyable flavor of foodstuffs and beverages. Therefore, the ability to distinguish bitter toxins from healthy bitter compounds would be useful. However, the discriminatory power of the sense of taste for bitter compounds appears to be limited and is disputed. In mammals, bitter taste is mediated by a special population of oral receptor cells dedicated to detect the numerous bitter compounds by means of ~30 Tas2r receptors. Discrimination of bitter compounds by the taste system would require that these receptor cells are functionally distinct based on the coexpression of different subsets of Tas2rs rather than the entire repertoire. We demonstrate by PCR that mice with genetically ablated Tas2r131 bitter receptor cells and extinguished Tas2r131 RNA levels still express several other Tas2rs. *In situ* hybridization also reveals that the animals still possess numerous taste cells characterized by robust expression of Tas2rs. Moreover, the mice show functional responses to many but not all bitter stimuli. Together, our data clearly demonstrate that bitter receptor cells are heterogeneous mediating distinct responses to bitter stimulation.

#P22. Involvement of T1R-independent receptors in detection of umami taste in mice

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Umami, typically elicited by monosodium l-glutamate (MSG), is known as one of five basic taste qualities. Molecular studies have identified multiple potential umami receptors, such as T1R1/T1R3, mGluR1 and mGluR4.

Previous studies with T1R-KO mice reported some controversial results. One study showed that behavioral and chorda tympani (CT) nerve responses to umami alone, and both umami and sweet compounds were abolished in T1R1-KO and T1R3-KO mice, respectively, whereas another study showed largely reduced, but not abolished, behavioral and CT responses to umami in another T1R3-KO strain of mice. The latter study suggests that multiple receptors contribute to umami taste. To further test this possibility, we newly generated T1R1-KO mice and examined their behavioral and CT and GL responses to umami and other tastes and potential effects of antagonists of mGluRs (AIDA for group I mGluRs; CPPG for group III mGluRs) on the responses. The results showed that T1R1-KO mice exhibited complete loss of synergistic responses to MSG and IMP but substantial residual responses to MSG alone in both CT and GL nerves. MSG responses were significantly inhibited by addition of AIDA or CPPG in both T1R1-KO and wild-type mice. Behavioral experiments, by using a conditioned taste aversion test, demonstrated that similar to wild-type littermates, T1R1-KO mice can discriminate MSG from other basic taste stimuli, and the avoidance conditioned to MSG was significantly reduced by addition of AIDA or CPPG in a concentration-dependent manner. These results suggest that mGluRs may be involved in taste responses to MSG in mice.

#P23. *Kalopanax pictus* sprout, a wild edible plant selectively activate hTRPA1

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Kalopanax pictus Nakai (Araliaceae) sprout is a wild edible plant widely used in Korea for its unique somatosensory properties. However, the neural receptors responsible for the pungency of this plant have not been identified yet. The TRPA1, which is expressed in the nociceptive neurons, induce a sensation of heat on activation by some pungent ingredients in food. We performed calcium imaging analysis using a human TRPA1 stably expressing HEK293 cells to test the interaction with the plant and TRPA1 receptor. The ethanolic extract of *Kalopanax pictus* sprout and its aqueous fraction increased intracellular Ca^{2+} concentration, which was virtually abolished by ruthenium red, a general TRP antagonist. The selected compositions of *Kalopanax pictus*, chlorogenic acid, protocatechuic acid, methyl syringate, alpha-hederin, and hederacoside C were examined the effects on hTRPA1. Protocatechuic acid, hederacoside C and methyl syringate activated hTRPA1. Methyl syringate, a low molecular weight phenolic ester shows the strongest effect of these compounds. The potency of activation by methyl syringate (1 mM) was almost equal as that of allyl isothiocyanate (50 uM), which is known as the most potent TRPA1 agonist among all natural products. Ruthenium red completely eliminated this response. These results demonstrate that food-derived natural compounds can activate human TRPA1 and suggested *Kalopanax pictus* Nakai (Araliaceae) sprout primarily activate TRPA1 to induce its unique pungency.

This study was supported by Korea Food Research Institute grants E0111501 and ER101000.

#P24. Taste perception in a praying mantid

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The visual and auditory systems of praying mantids have been well-studied, but much less is known about their chemosensory systems. In a series of feeding trials, we examined the sensitivity of giant asian praying mantids (*Hierodula membranace*) to different concentrations of bitter (quinine and denatonium) and sweet (sucrose and glucose) solutions. Using the length of time the mouthparts moved after being fed a 2μl drop of each solution, we were able to assay the sensitivity of the predators to the tastants. We also presented bitter substances on their forelegs, but did not measure any response that suggested that they could taste the solutions. To our knowledge, these are the first systematic investigations into taste perception in this insect predator, and enable us to start to understand how taste is used in predatory decisions.

This study was supported by a Nuffield Vacation Scholarship.

#P25. Expression and biophysical characterization of the human T1R1 taste receptor subunit

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The umami taste receptor is a heterodimeric receptor composed of the T1R1 and T1R3 subunits. These subunits belong to the family of class C G protein-coupled receptors (GPCRs) and are constituted by a large N-terminal (NTD) linked to the transmembrane domain by a cysteine-rich region. The umami taste of L-glutamate (L-Glu) can be potentiated by 5' ribonucleotides (IMP, 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 close to the opening and stabilise the T1R1-NTD closed conformation (Zhang et al, 2008). To further understand the structural basis of umami stimuli recognition by the T1R1-NTD, a large amount of purified proteins is required for biochemical and structural studies. In the present study, we report the successful expression and purification of the soluble T1R1-NTD. First of all, the protein was overexpressed as insoluble aggregated protein (inclusion bodies), using *Escherichia coli*. T1R1-NTD was then solubilised and *in vitro* refolded using suitable buffer and additives. Circular dichroism and fluorescence spectroscopy demonstrated that T1R1-NTD is correctly refolded. Owing to the large amount of produced protein, T1R1-NTD binding properties have been investigated using isothermal titration microcalorimetry. Microcalorimetry experiments demonstrated that T1R1-NTD is able to bind L-Glu and IMP with physiological relevant affinity. In summary, our expression system will enable large scale production of active protein suitable for crystallographic studies.

Zhang et al (2008) Proc Natl Acad Sci U S A, 105, 20930–20934.

This work was supported by INRA and Burgundy council (Région Bourgogne).

#P26. Chemoreception and feeding behaviour in the red swamp crayfish Procambarus clarkii

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The red swamp crayfish Procambarus clarkii (Girard, 1852) (Crustacea: Decapoda) is a invasive species that, since its use in aquaculture practices, has spread worldwide.

We investigated the behavioral feeding responses in relation to the sensitivity of the walking legs of P. clarkii to a few food components for such an omnivorous species, like the carbohydrates maltose, sucrose, trehalose and the amino acids glycine, leucine.

The feeding response was determined using a 4-level ranking score, in an intact animal bioassay including 1) antennular flicking, 2) movement of the walking legs, 3) the activity of maxillipeds and 4) the animal's attempt to access the stimulus-containing area. The extracellular spike activity from fascicles of pereopod nerves was recorded by way of conventional suction electrodes.

Our behavioural results show that 1) none of the tested stimuli specifically activates antennular flicking, 2) maltose, leucine and glycine elicit different qualitative response from crayfish, with the first two compounds being effective for the walking legs and the last almost exclusively for maxillipeds, 3) sucrose and trehalose activate both appendages, but only at the highest tested concentration, 4) the strongest behavioral level, the animal's attempt to access the stimulus-containing area, is usually coupled to an increase of the walking leg rather than of the maxilliped activity. Electrophysiological experiments on pereopods confirm the behavioral observations.

Activity of antennules vs. walking legs and maxillipeds of the crayfish in relation to food search is discussed in the light of the development of effective strategies for population control programmes.

#P27. Taste response profiles change in relation to nutritional history in two closely related species of Lepidoptera

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The spike activity of the maxillary taste chemosensilla in the larvae of two related species of Lepidoptera (*Papilio machaon* L. and *Papilio hospiton* Géné) raised on different host plants, was recorded with electrophysiological techniques after stimulation with NaCl, sucrose and host plant extracts, with the aim of cross-comparing their response patterns and evaluating any effects of different feeding histories. The spike discharges were analysed and the firing neurons were sorted by means of a specific software (SAPID Tools). The discrimination capabilities and modalities in the taste systems of the two species were measured and confronted by calculating vector space analysis parameters (delta and lambda). The results show that: a) all 4 chemosensory cells in lateral and medial sensilla of both species responded to each stimulus; b) *P. machaon* larvae raised on ferula exhibited response spectra and discrimination profiles somewhat intermediate between those of *P. machaon* on fennel and of *P. hospiton* on ferula, the latter two presenting a higher degree of difference; c) in the time course analysis of the spike discharges, the highest similarity was found between spike activities of *P. hospiton* and *P. machaon* raised on the same host plant; d) for both species, the coding modality involved in the detection of both pure stimuli (salt and sugar) and complex ones like host plant extracts, is mostly an "ensemble code" of the across-neuron pattern type. The data support the hypothesis that diet-related factors may influence peripheral chemosensitivity in lepidopterous larvae, inducing functional shifts that partly compensate genetic divergence.

#P28. Genetic tracing originating from Type II taste receptor cells does not reveal gustatory pathways

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Towards detailed understanding of gustatory information processing we intended to visualize gustatory neurons linked to bitter or umami taste by manipulating mice to express the transsynaptic tracer barley lectin and hrGFP or mCherry fluorescence proteins in the loci of the Tas2r131 bitter receptor and the umami receptor subunit Tas1r1, respectively. Faithful expression of the knockin alleles enabled us to visualize and distinguish tracer source from recipient cells. In both models, barley lectin associates with all taste bud cell types. Therefore, the observed lectin-labeled fibers likely received the tracer directly from the source cell as well as indirectly following lateral diffusion preventing identification of taste-specific fibers. Moreover, the number of lectin-labeled neuronal somata in the ganglia disagrees with that of connected receptor cells. In addition, although numerous neurons in various gustatory areas contain the tracer, the first order central taste neurons do not or only rarely. Even though we did not detect fluorescent cells and Tas2r131 and Tas1r1 expression by *in situ* hybridization outside the taste buds, we suspect that the extra-taste bud lectin originates from local low level gene expression. To follow up this suspicion we engineered mice to efficiently and faithfully express barley lectin and red fluorescence protein from the Rosa26 locus in Tas2r131 and Tas1r1 cells. This strategy eventually identified Tas2r131 and Tas1r1 expressing cells in taste buds, the gustatory ganglia, brain stem and various other gustatory and non-gustatory brain areas. Together our data demonstrate that gustatory pathways cannot be traced reliably from Type II taste receptor cells.

#P29. Expression analysis of Gα subunits in sweet and umami taste cells by single cell RT-PCR

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T1Rs are G-protein coupled receptors that mediate sweet (T1R2/T1R3) and umami (T1R1/T1R3) taste in taste receptor cells. Tastant binding to sweet and umami receptors activates heteromeric G protein including α-gustducin, and subsequent stimulation of phospholipase Cβ2, production of IP_3, an increase in $[Ca^{2+}]_i$, and activation of TRPM5. Previous studies demonstrated that mice lacking α-gustducin gene showed diminished but not abolished neural and behavioral responses to sweet and umami substances, suggesting the possibility that other Gα subunits contribute to sweet and umami taste responses. In this study, we identified T1R3 expressing cells using transgenic mice, which express green fluorescent protein under control of T1R3 promoter, and examined the expression of Gα subunits (11, 12, 13, 14, 15, i1, i2, i3,t1, t2, s, q, o, z, olf, and gustducin) in these cells by using multiplex single cell RT-PCR. Many T1R3-GFP cells expressed Gα11, Gα14, Gαi2, Gαq, Gαs, and Gagust. Expression of several Gα subunits such as Gαolf, Gαz, Gαo, Gα15 were rarely detected in T1R3-GFP cells. These results raise the possibility that Gα11, Gα14, Gαi2, Gαq, and Gαs may contribute to sweet and umami transduction in taste cells. In addition, the number of T1R3 GFP taste cells expressing gustducin and Gα14 were significantly different between fungiform and circumvallate papillae. These differences may contribute to topographical differences in sweet and umami sensitivity on the mouse tongue.

This study was supported by KAKENHI 23089076 (R.Y.) and 23249081 (Y.N.).

#P30. Type II and III taste bud cells expressed BDNF and NT3 in rat

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Taste is an essential sense to survival and growth of animals, and the growth and maintenance of taste organs are under regulation of various neurotrophic factors, but distribution aspect of neurotrophic factors and their receptors in distinct taste cell types is not clear.

The present research was designed to characterize mRNA expression pattern of neurotrophic factors and their receptors in distinct type of taste cells. In Sprague-Dawley rats, epithelial tissues which did not include or included circumvallate and folliate papillaes were dissected, homogenized, and mRNA expressions for neurotrophic factors and their receptors were determined by RT-PCR. The mRNA expressions of BDNF, NT3, TrkB, but not NGF, NT4/5, TrkA, TrkC, p75NGFR, were observed in some population of taste cell. Moreover, to characterize which type of taste cells express NT3, BDNF, or TrkB, we examined mRNA expressions of NT3, BDNF, or TrkB in the PLCβ2(a marker of Type II cell)- and/or SNAP25(a marker of Type III cell)-positive taste cells by a single taste cell RT-PCR. Subsets of taste cells expressed NT3, BDNF, and TrkB as 84.1, 70.3, and 1.4 %, respectively. In addition, all of PLCβ2- and SNAP25-positive taste cells expressed NT3 mRNA, except for a taste cell. The expression ratios of NT3 mRNA were 100 and 91.7% in the SNAP25- and PLCβ2-positive taste cells, respectively. However, two TrkB-positive taste cells co-expressed neither PLCβ2 nor SNAP 25.

The results suggest that the most of type II or type III cells express BDNF and NT3 mRNA, but less in type I taste cells.

This study was supported by Basic Science Research Program through the NRF in Korea funded by MEST(2010-0007135).

#P31. Selective gating of mating male chemosignals and neural responses to MHC peptides following mating in mice

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Female mice form a memory to their mate's urinary chemosignals at mating, which prevents them from eliciting the pregnancy block effect. This mate recognition is proposed to involve a learning-induced increase in feedback inhibition, which selectively gates the transmission of the learned signal at the level of the accessory olfactory bulb (Brennan, 2009). We have used immunohistochemistry for the activity marker c-Fos and tyrosine hydroxylase to identify dopaminergic cells at the neuroendocrine output, in the arcuate nucleus of the hypothalamus, which are activated by male bedding exposure. We found a significantly lower activation of arcuate dopaminergic neurons in mated female mice in response to bedding from the mated male than to bedding from an unfamiliar male. The strain specificity of urinary chemosignals that underlies recognition of the mating male is conveyed by nine amino acid peptides

ligands of major histocompatibility complex (MHC) class I proteins (Leinders-Zufall et al., 2004). In a separate experiment, we recorded single unit activity from the medial amygdala of awake, behaving mice that had been mated with either BALB/c or C57BL/6 males. Spike rate was significantly lower in response to exposure to bedding from the mated male than to bedding from an unfamiliar male, or to bedding from the mating male that had been sprinkled with MHC peptides of unfamiliar strain type. These results are the first demonstration of a neural response to MHC peptides in vivo and are consistent with the gating hypothesis for mate recognition.

Brennan (2009) Behav. Brain Res., 200, 287-249; Leinders-Zufall, et al. (2004) Science, 306, 1033–1037.

This work was supported by BBSRC grant BB/C005015/1. The MHC peptides were a gift from Thomas Boehm, Max Planck Institute of Immunobiology, Freiburg, Germany.

#P32. Puberty acceleration in female mice: behavioural effects and neuroendocrine consequences

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In mice, male urinary pheromones are known to accelerate female puberty onset, however, the neurobiological regulations as well as the behavioural consequences of this exposure are still unknown. When exposed from weaning (*i.e.* 21 days) to male soiled bedding, juvenile females show an earlier vaginal opening and a increased vaginal weight in comparison to control females exposed either to clean bedding or bedding soiled by castrated males. After the end of the pheromonal stimulation, we tested its impact on subsequent mate preference at the age of 45 days. In Y-maze tests between bedding odours derived from sexually active males over those derived from receptive females or castrated males, females exposed to male bedding showed a strong preference for the odour of the male. This result suggests that, in addition to the effects observed on reproductive function, peripubertal exposure to male pheromone may also have enduring effects on subsequent sexual experience. Given that the *Kiss-1* gene is mandatory for puberty onset, we also hypothesised that the peripubertal exposition to male olfactory cues could have some impact on the architecture of the Kisspeptin system (number of Kisspeptin neurons and/or of Kisspeptin appositions on GnRH neurons) which is known to develop juste before puberty onset. By using double immunocytochemistry and confocal microscopy, we are now characterising the architecture of the Kisspeptin system in the the hypothalamic regions containing Kisspeptin and/or GnRH neurons. This approach should tell us whether peripubertal exposure to male pheromones targets Kisspeptin neurons to impact the gonadotrope axis and accelerates puberty.

This study was supported by the french ANR Jeunes Chercheurs program (ANR PHEROSEX).

#P33. L-felinine as a potential chemical signal in the house mouse

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Felinine is a unique sulfur-containing amino acid found in the urine of domestic cats. In our earlier studies we showed that exposures of mice to the urine from feral cats significantly affected survivorship of offspring. Dietary manipulations with urine donors revealed the key role of sulfur-containing compounds. In current study we examined the influence of the precursor of potential Felidae family pheromone L-felinine on reproductive output in mice. We used three basic approaches: behavioral, endocrinological and immunohistochemical. We recorded number of newborn pups, sex ratio, weight of the pups at the weaning. Corticosterone metabolites were monitored non-invasively (Touma et al., 2003). Fos-positive cells were recorded in response to stimulation with L-felinine in the main olfactory (MOB) and accessory olfactory bulbs (AOB) and in the vomeronasal receptor tissue. Cotton balls soaked with felinine (0.05% w/v, 0.05 ml) were placed into home cages of mice during all period of gestation. Control animals were exposed to tap water. Exposure to L-felinine affected sex ratio in mice ($n=40$, $p<0.001$) in favour of males. L-felinine also affected litter size ($n=40$, $p<0.05$). By the day of weaning in control groups of animals average weight of pups was significantly ($p<0.001$) higher then in the exposed to L-felinine. We observed long lasting elevation of corticosterone under L-felinine exposure ($n= 10$, $p<0.001$). Immunohistochemical studies showed Fos-immunoreactivity in the MOB and AOB indicating the involvement of both systems in the detection of L-felinine. The data obtained indicate that L-felinine is a potential heterospecific chemical signal affecting reproduction in mice.

Touma, C, Sachser,N., Mostl, E., and Palme, R. (2003). Effects of sex and time of day on metabolism and excretion of corticosterone in urine and feces of mice. General and Comparative Endocrinology. 130, 267-278.

This study was supported by RFBR 10-04-01599 to VVV.

#P34. Pheromone detection in *Heliothis virescens*

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In many moth species the males can accurately detect very low concentrations of female-released sex pheromones. This remarkable ability is based on specifically tuned olfactory "hairs" on the antennae. These sensilla trichodea house the dendrites of olfactory sensory neurons each endowed with distinct pheromone receptors in their membranes. The dendrites are bathed in aqueous sensillum lymph containing pheromone binding proteins (PBPs), which are secreted from support cells and are supposed to mediate the transfer of pheromones towards their receptors. *Heliothis virescens* females release a pheromone blend for mate attraction, with Z11-hexadecenal as the major component. Previously, we have cloned several candidate pheromone receptors expressed in the antenna of male *H. virescens.* Using heterologous expression systems, we found that the receptor type HR13 was activated by Z11-hexadecenal. In addition, PBP2, but not PBP1 could mediate a response of HR13-expressing cells to the pheromone. FISH-experiments on sections of male antennae revealed that PBP2 is expressed by support cells, while the HR13-expressing neurons also express the "sensory neuron membrane protein 1" (SNMP1) and the olfactory co-receptor Hvir\Orco. Our results suggest that PBP2, HR13, SNMP1 and Orco

interplay in the detection of the major sex pheromone component. To explore the specific roles of the identified proteins, attempts were made to further reconstitute the pheromone detection machinery in heterologous systems. Moreover, to approach a possible interference of the pheromone detection system with odorants coexisting in the environment of calling females, we have analyzed Z11-hexadecenal binding to PBP2 and HR13 in the presence of plant-derived odorants.

This work was supported by the Deutsche Forschungsgemeinschaft SPP 1392.

#P35. Loss of aggressiveness and response to pheromones in mice lacking the G-protein γ8-subunit

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G-proteins are strongly involved in the transduction mechanisms underlying odorant and pheromonal signaling. The complementary expression of two specific G-proteins α-subunits, Gαi2 and Gαo, defines a basal/apical partition of two chemosensory neuron subsets of the vomeronasal organ. In many biological systems, G-protein signalling is also modulated by the activity of Gβγ subunits of this heterotrimeric complex. In the vomeronasal organ, two different G-protein complexes have been characterized. Gβ2γ8 is preferentially expressed in the basal neurons whereas Gβ2γ2 in the apical ones. Here we have investigated the physiological effect and pheromonal responses in mice with a targeted deletion of the Gγ8 gene. The accessory olfactory organ appears normal at birth but undergoes a great loss of basal neurons starting from postnatal day 28. This loss is associated to a diminished protooncogene expression in the accessory olfactory bulb of pheromonally stimulated mice. Noteworthy, Gγ8 deletion led to a reduced aggressiveness in both males and females. Thus, the present study suggests a central role of the olfactory specific G-protein γ-subunit, Gγ8, in the pheromonal transduction mechanisms.

#P36. Next Generation Sequencing Reveals Variation in Vomeronasal Receptor Gene Repertoires across 17 Mouse Strains

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Innate behaviour in mammals, including aggression, sex and parenting are largely instructed by chemoreception: signaling pheromones detected by vomeronasal receptors (VRs). As inbred strains of mice display stereotypical differences in such behaviour, we hypothesize that variation in VR genes may underpin these phenotypes. Here we report the single nucleotide polymorphism (SNP) content of 17 mouse strains, in over 3,000 VR genes.

16-34x sequence coverage was generated for each strain using the Illumina platform, producing a genome-wide call set of more than 65 million SNPs. Our analysis found over 6,500 coding variants in VR genes. As expected, wild-derived strains have an order of magnitude more variation than classical lab strains. We identified potentially truncating VR mutations, and found that the functional receptor repertoire between different species and sub-species of mice may differ by over 10%. Moreover we found clear evidence of duplications and deletions of some VRs, suggesting that strains, species and sub-species differ in their ability to sense pheromones.

Although the number and distribution of SNPs vary among VR genes, the mean rate of SNP accumulation is similar between VR subfamilies. However principal component analyses reveal correlations within chromosomal loci and phylogeny within subfamilies, suggesting VR clusters may delineate conserved functional units in pheromone detection. This is particularly evident in classical laboratory strains and may be a consequence of artificial selection for or against some innate behaviours during domestication. These data, combined with ongoing analysis of variation in VR expression, will assist in assigning function to the vomeronasal receptor system.

This study was supported by The Wellcome Trust, grant 093865/Z/10/Z. We are grateful to the Mouse Genomes Project for sharing prepublication sequence data.

#P37. Anatomical study on olfactory apparatus of *Lepidocephalichthys guntea* (Hamilton, 1822)

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Lepidocephalicthys guntea is a (Teleostean: Cobitide) bottom dwelling fish in South East Asia. The preliminary anatomical work was held in laboratory using aqueous Bouins solution as fixative in a normal room temperature and viewed under optical binocular microscope. Preliminary anatomical observation reveals that the olfactory structure is associated with olfactory rosette, accessory nasal sacs (?), olfactory nerve tract, olfactory bulb and brain (Doving et al, 2007; Sarkar et al, 2011). The olfactory rosette as a whole is a semielliptical structure in shape. The temporary glycerin preparation shows that the rosette shows different shapes of lamellae radiating from the central axis. We assume that this apparatus (rosette) is the key region for detecting the chemical cues of aquatic environment. This observation is to be confirmed by advanced microscopical study with reference to enzymhistochemical reactions. This work is under progress.

Doving et al. (2007). Prog. Neurobiol., **82** : 80–86; Sarkar et al.(2011) Int. J.Sc.Nat., **2**(1):1–6.

Authors are thankful to S.K. Sarkar, JRF, Department of Zoology, Vidyasagar University for his necessary help.

#P38. Search of ligands for the unique OR37 odorant receptors

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The capacity of the mammalian olfactory system to detect an enormous array of different chemical compounds is based on a large repertoire of odorant receptors (ORs). A small group of these ORs, the mOR37 family, is unique due to a variety of special

features. Members of the OR37 family are exclusively found in mammals, they share a high degree of sequence homology and are highly conserved during evolution. It is still elusive which odorants may activate these atypical receptors. We have reasoned that compounds from skin, hairs or skin glands might be potential candidates. Such compounds are mostly very hydrophobic and therefore difficult to solubilize for analysis in heterologous expression systems. As an alternative approach we have exposed mice to such compounds and monitored activation of glomeruli through the expression of the activity marker c-fos in juxtaglomerular cells surrounding ventrally positioned glomeruli in the olfactory bulb (OB). Employing this methodology it was found that stimulation with long-chain alkanes elicits activation in the ventral part of the OB; however, none of the OR37 glomeruli. Analyses of long-chain hydrocarbon compounds with different functional groups revealed that long-chain aliphatic aldehydes elicited an activation of OR37A or OR37B glomeruli; each of them responding preferential to an aldehyde with different chain length. The OR37C glomerulus did not respond. These results indicate that OR37 receptors may be specifically tuned to distinct long-chain aliphatic aldehydes with a significant degree of ligand specificity.

This study was supported by Deutsche Forschungsgemeinschaft (DFG).

#P39. Voltage and calcium regulation of anoctamin2/TMEM16b: the calcium-activated chloride channel involved in olfactory transduction

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Olfactory transduction involves a depolarizing chloride current activated by intracellular calcium. Indeed, the binding of odorants to receptors in the cilia of olfactory sensory neurons activates a cAMP-mediated signalling pathway which triggers a Ca^{2+} influx. The increased intracellular Ca^{2+} subsequently opens Ca^{2+}-activated Cl^- channels (CaCCs) which produce an efflux of Cl^-. Recent studies indicate that anoctamin2/TMEM16b is the channel involved in olfactory transduction. Indeed, TMEM16b is expressed in the membranes of olfactory cilia, has channel properties similar to native olfactory CaCCs, and olfactory sensory neurons from knockout mice for TMEM16b$^-$ do not have any Ca^{2+}-activated Cl^- current. Channel gating is both Ca^{2+}- and voltage-dependent. Although TMEM16b does not have any apparent canonical Ca^{2+}-binding site, the first intracellular loop contains 5 consecutive conserved glutamic acid residues. Those residues have been deleted and the functional properties of wild-type (wt) and mutant channels were compared using the whole-cell voltage-clamp configuration. Cells were dialyzed with intracellular solutions containing various amounts of Ca^{2+} and currents were measured using a voltage step protocol from -100 mV to +100 mV. The dose-response relationship of wt channel shows an EC_{50} at +60 mV of 2.0 ± 0.2 μM and at -60 mV of 6.3 ± 1.3 μM. The mutant has an EC_{50} at +60 mV of 2.8 ± 0.2 μM and at -60 mV of 3.8 ± 0.6 μM. Removing the 5 glutamic acid region reduces the voltage-dependence for EC_{50} and the activation curve is shifted toward higher voltages. These results indicate that the region rich in glutamic acid residues located the first intracellular loop is involved in channel gating.

#P40. The effect of perinatal naris occlusion on nasal turbinate development: Does emmetropization occur in the nose?

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Unilateral naris occlusion, a standard method for producing stimulus deprivation in the olfactory system, causes marked airflow changes in both the occluded and open nasal cavity. While on the occluded side the airflow is dramatically reduced, especially rostrally, the open side is forced to carry a larger than normal volume. Also, naris occlusion abrogates alternating cycles of breathing, forcing constant duty on the open side. We were interested if these changes in airflow effect development of nasal turbinates, particular given the dearth of information on their ontogenesis. We therefore investigated mice aged 18-25 days, that had been naris occluded or shame operated on the day of birth. Coronal serial sections were examined throughout the rostrocaudal extent of the nasal cavity for turbinate structure. Results demonstrate that naris occlusion has signficant effects on the size, shape, and position of nasal turbinates, especially rostrally. The most anterior turbinate, endoturbinate-I, takes on a delicate ''filigree'' appearance on the occluded side relative to the open side: 24% decrease in area/perimeter (open 65.2, closed 52.6; control 59.5 $\mu m^2/\mu m$; $p<0.005$) despite same perimeter; 82% increase in length/width, (open 13.5; closed 7.4; control 7.9 μm/μm; $p<0.001$). That these effects are attributed to airflow is supported by the intermediate values of controls. We conclude that a stimulus from respiratory airflow: mechanical, thermal or chemical, causes epigenetic changes in the ontogeneis of nasal turbinate structure. These observations raise the intriguing possibility that, as in the eye, the turbinate system in the nose may develop by environmentally guided emmetropization.

This study was supported by National Science Foundation grant #0641433 to DC

#P41. Evidence for multiple interactions at the olfactory receptor level

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Most of naturally occurring odours arise from the perception of complex mixtures of volatile compounds. Therefore perceptual properties (intensity and quality) of odorant mixtures depend on interactions between odorants and olfactory receptors as well as olfactory signal processing in the olfactory bulb and in the central

nervous system. There is increasing evidence that peripheral processes play an important role in mixture perception. Indeed, competitive and non-competitive interactions have been observed at the olfactory sensory neuron level.

The present study aimed to explore the activation of olfactory receptors (OR) by binary mixtures of odorants. We were particularly interested in an odorant couple present in red wines, isoamyl acetate (IAA) and whiskey lactone (WL), which has been shown to induce masking or synergy according their ratio in mixture. For this study, we used a heterologous expression system (HEK293T cells) in which OR (OR1G1 and OR52D1) were transfected transiently. Responses of OR to odorants applied alone or in combination were measured by calcium imaging.

The results showed that both OR are activated by IAA and WL. When these molecules are used in combination, phenomena of compromise, subtraction and partial addition) have been identified, depending on the OR and the concentrations of compounds in the mixture. These observations suggest that the sensory image of a mixture is not encoded through the sum of the codes of each component, especially because interactions occur at the peripheral level.

#P42. Prion protein deficient mice show no alteration in olfactory-guided behavior

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The absence of the prion protein (PrP^C) in neurons has been shown to induce subtle alterations in olfactory-guided behavior and modifications in olfactory bulb activity, suggesting that PrP^C may modulate the olfactory processing (Le Pichon et al, 2009). In light of this data, we have analyzed the dependence of the olfactory behavior on PrP^C using mice with another genetic background, i.e., we compared wild-type (WT) (FVB strain), congenic PrP-knockout (KO, line F10) and PrP-rescued (TG46) 3 month-old male mice (n=8 in each group), singly housed at the time of testing. Different behavioral domains were tested: motor performance and learning (pole test, repeated in 3 consecutive days), exploratory activity and locomotion (open field test), intraspecific aggressive behavior (intruder test), emotional reactivity in a hostile environment (forced swimming test) and olfactory-guided behavior (cookie-finding test). In the pole test, PrP-KO mice constantly performed better than the other two groups, while in the forced swimming test the latency to the first stop was longer for KO mice compared to the WT counterparts. Conversely, no difference was detected among the different murine lines neither in the open field and intruder tests, nor during the 2 consecutive olfactory- and the 3rd visually-guided search of the cookie-finding test. In conclusion, the results obtained in our model animals suggest that the absence of PrP^C does not necessarily generate olfactory deficits, in line with recent investigations on the activity of the olfactory epithelium (Dibattista et al, 2011).

Le Pichon et al (2009) Nat. Neurosci., 12, 60-69; Dibattista et al (2011) Chem Senses. 2011 Jun 16. [Epub ahead of print].

Study supported by the University of Padova.

#P43. A role for apical translation in the control of olfactory mucosa survival?

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In mammals, olfactory sensory neurons (OSN) are located at the interface between environment and the brain for proper odorant coding by the olfactory mucosa (OM). These neurons have developed specialized structures, the olfactory dendritic knobs and cilia that contain elements of the olfactory transduction pathways. OSN are surrounded by several cell types bearing microvilli at their apex, such as glia-like sustentacular cells (Sus).

By its location between the environment and the brain, the olfactory mucosa (OM) undergoes several external aggressions for which it has developed original defense strategies. Recent demonstration of the existence of a local transcriptome in the apical region of the OM prompted us to postulate the local translation of mRNAs in dendrites. The fine examination of OM apical region using both structural and ultrastructural immunohistochemical tool revealed the presence of several proteins and rRNAs involved in the mRNAs transport and translational processing. Moreover, we were able to purify polysomes from dendritic knobs-enriched preparations. All these data provide strong evidence for a local translation of mRNAs in the apical part of the OM, ie in olfactory neurons dendritic knobs and in surrounding cells apex (Sus).

#P44. Adaptive bias in the response of the ON and OFF olfactory receptor neurons to creeping changes in food odor concentration

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Extracting temporal information from odor signals is an important component to the functioning of many different olfactory systems. For walking and flying insect, this information is critical for orientation to odor sources. The cockroach *Periplaneta americana* and the moth *Agrotis ipsilon* receive information about the odor of lemon oil via parallel ON and OFF olfactory receptor neurons (ORNs), which provide excitatory responses to concentration increments and decrements. By utilizing a ''push-pull'' arrangement, each ORN amplifies the opposite halves of the input signal, which yields excellent efficiency for encoding fluctuations in odor concentration.

In both insect species, however, the ON and OFF ORNs are not symmetrical systems with equal and opposite responses: the OFF ORNs display higher discharge rates to drops in odor concentration than the ON ORNs to concentration jumps (Tichy et al., 2005; Burgstaller and Tichy, 2011; unpublished data). Here we focus on the responses of the ON and OFF ORNs to continuously rising concentrations (up-ramps) and continuously falling concentrations

(down-ramps). We found that in both insect species the OFF ORNs display higher discharge rates to down-ramps than the ON ORNs to equivalent up-ramps. The results suggest that response biases to falling versus rising concentration reflect an adaptive advantage for both walking and flying insects – such as economy of movement and likelihood of loosing the odor signal. The bias for continuously falling concentration overestimates the disappearance of the odor signal, thereby providing a margin of advanced safety during the search for food resources.

Tichy H, Hinterwirth A, Gingl E (2005) Olfactory receptors on the cockroach antenna signal odour ON and odour OFF by excitation. European Journal of Neuroscience 22:3147-3160.

Burgstaller M, Tichy, H (2010) Functional asymmetries in cockroach ON and OFF olfactory receptor neurons. J. Neurophysiology 105:834–845

This work was supported by Austrian Science Fund Grant P 21777-B17.

#P45. Role of xenobiotic metabolizing enzymes in the perception of caffeine in Drosophila melanogaster

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Detoxification and elimination of potentially harmful chemicals are crucial processes for most organisms. Xenobiotics Metabolizing Enzymes (XME: CYP, UGT···) are able to take in charge exogenous molecules, biotransform and eliminate them out of the organism. In addition to a high expression in fat body or malpighian tubules, XME have been detected in the chemosensory organs where they may modulate chemoperception by neutralizing the stimulus molecule after detection, avoiding receptors saturation and readying the neuron to respond to another stimulus. The aim of this study is to investigate the potential role of cytochromes P450 (CYP) in the perception of caffeine in *Drosophila melanogaster*. We showed that the expression of several CYP, expressed in chemosensory organs, is up- regulated after an exposure to caffeine in adult flies. This result suggests that these CYP genes could be involved in the metabolism of this component. We hypothesized that a modulation of CYP expression should provoke a modification of caffeine detection and alter animal feeding behaviour. To confirm this hypothesis, we targeted the inhibition of candidate CYP genes expression in sensory organs using interference RNA (RNAi). We evaluated the ability of these transgenic flies to detect caffeine using a behavioral test (MultiCAFE), which allows to estimate the consumption of caffeine solution. We noted that a reduction of the expression of these CYP genes disturbs feeding behaviour. RNAi-CYP animals fed significantly more than controls on food containing caffeine, which is normally repellent. These data suggests, for the first time, that some CYP genes, expressed in sensory organs, may be involved in caffeine chemoperception in Insects.

#P46. Two odorant-binding proteins mediate response to the alarm pheromone (E)-β-farnesene in aphids and suggest a new strategy to identify aphid repellents

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Aphids are agricultural pests of great economical interest. As alternative to insetticides, the use of semiochemicals, like sex pheromone and alarm pheromone, meets some difficulties in these insects. The sex pheromone is of little practical use in aphids that reproduce mostly parthenogenetically. The alarm pheromone, (E)-β-farnesene for most aphid species (Bowers et al, 1972; Pickett et al, 1980; Francis et al, 2005), is not persistent in the environment and its chemical synthesis is complicated and expensive. Our objective is the search for novel semiochemicals to be used in population control through the study of the olfactory, especially of odorant-binding proteins (OBPs) that have been shown to play a central role in olfactory recognition (Xu et al, 2005; Pelosi et al, 2006; Matsuo et al, 2007). We have tested 29 compounds, including (E)-β-farnesene, in binding assays with 6 recombinant OBPs and in behaviour experiments. We have found that good repellents bind to OBP3 and/or OBP7, while non repellents present different spectra of binding. These results have been verified with two species of aphids, *Acyrthosiphon pisum* and *Myzus persicae*, both using (E)-β-farnesene as the alarm pheromone. Our results support the idea that OBPs are involved in decoding the chemical information of odorants and pheromones and also offer guidelines for the discovery of potential alarm pheromones.

Bowers et al (1972) Science, 177, 1121–1122; Pickett et al (1980) J. Chem. Ecol, 6, 349–360;

Francis et al (2005) J. Appl. Entomol, 129, 6–11; Xu, P et al (2005) Neuron, 45, 193–200;

Pelosi et al (2006) Cell. Mol. Life Sci, 63, 1658–1676; Matsuo et al (2007) PLoS Biol, 5, 985–996.

#P47. The Odorant-binding Proteins of *Anopheles gambiae*

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Odorant-binding Proteins (OBPs) of insects have recently been shown to play a central role in odour coding and recognition, thus becoming biomolecular targets for strategies of insect population control. Here we present the first investigation on the expression of OBPs of *A. gambiae* in antennae of both sexes, as well as in larvae and pupae, using different proteomic techniques.

The results can be summarised as follows:

1. In male antennae only OBP1 and OBP9 are expressed at fairly high leves, with traces of 7 other OBPs. In females 12 OBPs are relatively abundant, with traces of other members. These data

confirmed those obtained at the RNA level, except for OBP4 and OBP5, that we could not detect in our analysis. Therefore, we have produced both proteins in bacteria and raised polyclonal antibodies. Western blot analysis could not detect either of the two proteins in male nor in female antennae, confirming the results of mass spectrometry. We could not exclude, however, that these proteins could be produced in particular conditions, such as, for instance, after a blood meal.

2. The same analysis allowed us to detect the presence of four out of the seven Chemosensory Proteins (CSPs) predicted by the genome in the antennae of both sexes.
3. In both larvae and pupae, the dominant protein is OBP9, accompanied by small amounts of the CSP SAP1 in the larvae.
4. The relatively small number of OBPs expressed at the protein level makes feasible a complete characterisation of each of them. Such information will represent a solid basis for designing new mosquito repellents.

#P48. Crystal structure of odorant binding protein 4 from *Anopheles gambiae*

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Malaria is the most common tropical disease, caused by the parasite *Plasmodium falciparum* which is transmitted to humans by female mosquitoes, particularly *Anopheles gambiae*, during blood feeding. Hydrophobic odor molecules that enter in the sensillum lymph cavity are captured by the odorant binding proteins (OBPs) which mediate the first steps in olfactory signal transduction cascade. OBPs are considered as targets for structure-based rational approaches for the discovery of new repellents. Of the 60 OBPs encoded in the genome of the *A. gambiae*, AgamOBP1, 4 and 48, were suggested to be involved in human detection and can form heterodimers with novel binding specificities (Biessmann et al, 2005; Qiao et al, 2011). Among them, AgamOBP1 (Wogulis et al, 2006) was until now, the only protein of known structure.

Recently, we have determined the structure of AgamOBP4 at three different pH values. The structural analysis showed that pH induces conformational changes mainly in the loop regions (aa. 56-66, 78-82 and 118-125). Importantly, in acidic pH, the N-terminal helix (aa. 1-10) becomes flexible resulting in the widening of the mouth opening of the binding site. It is possible that pH changes are related to odorant release mechanism as it has been shown for other OBPs (Leal et al, 2005). Comparison with LUSH (the AgamOBP4 homologue from *D. melanogaster*) and AgamOBP1 revealed that despite their common structural fold, AgamOBP4 exhibits a binding cavity of quite different environment suggesting different odorant specificities. AgamOBP4 constitutes a novel target for structure based design of more efficient repellents.

Biessmann et al (2005) Insect Mol Biol, 14, 575-589; Qiao et al (2011) Cell Mol Life Sci, 68, 1799-1813; Wogulis et al (2006) Biochem Biophys Res Commun, 339, 157-164; Leal et al (2005) Proc Natl Acad Sci U S A, 102, 5386-5391.

*We acknowledge the late Dr. Harald Biessmann and Dr. Marika F. Walter (Developmental Biology Center, University of California), for kindly providing the AgamOBP4 gene. This work was supported by the European Commission for the FP7- HEALTH-2007-2.3.2.9 project "**ENAROMaTIC**" (GA-222927), the FP7-REGPOT-2008-1 project "**EUROSTRUCT**" (GA-230146) and the FP7-REGPOT-2009-1 Project "**ARCADE**" (GA-245866)". Work at the Synchrotron Radiation Sources, **MAX-lab**, Lund, Sweden and **EMBL Hamburg Outstation**, Germany, was supported by the European Commission for the FP7 Research Infrastructure Action "**ELISA**" (European Light Sources Activities).*

#P49. AgamOBP1 crystal complex with DEET: a new molecular target for the design of novel mosquito repellents

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Malaria is the most deadly tropical disease, responsible for 863.000 deaths in 2008 (WHO, 2010). It is transmitted to humans by hematophagous mosquitoes, particularly *Anopheles gambiae*, carrying the pathogen parasite *Plasmodium falciparum*.

Mosquito repellents are the most commonly used agents for prevention of infection by keeping infected mosquitoes away from their human targets. DEET (*N*, *N*-diethyl-*m*-toluamide) is the most widely used repellent worldwide, with a broad effectiveness against most insects (Moore SJ, 2007). However, health problems during long-term use and ineffectiveness a few hours after the application, indicates the necessity of new more effective repellents. Previous efforts to identify more efficient insect repellents were hindered by the absence of a known molecular target and were thus limited only to ligand-based QSAR approaches.

Recently we have reported the high-resolution structure of AgamOBP1-DEET complex which provides the first example of a repellent recognized by an odorant binding protein (OBP) (Tsitsanou et al, 2011). One DEET molecule (K_d=31.3±1.4 μM), is bound with high shape complementarity to each subunit at the dimer interface and exploits 60 van der Waals interactions and one hydrogen bond to a water molecule.

Moreover, we used molecular modeling to predict the binding characteristics of 29 repellents. Our predictions were further confirmed by fluorescent displacement assays indicating that structure-based design can provide promising leads for the development of novel repellents.

Given that it takes about 10 years to develop a new repellent, the rational structure-based design approach will play an increasing role in the search for more effective repellents.

World Health Organization (2010). Malaria fact sheet.WHO website [online]; Moore SJ (2007). *In Insect repellents: Principles, methods, and uses*, Debboun M FS, Strickman D (ed), pp 3–30, CRC, Boca Raton, FL; Tsitsanou et al (2011) Cell Mol Life Sci. (Epub ahead of print).

*We acknowledge the late Dr. Harald Biessmann and Dr. Marika F. Walter (Developmental Biology Center, University of California), for kindly providing the AgamOBP1 gene. This work was supported by the European Commission for the FP7- HEALTH-2007-2.3.2.9 project "**ENAROMaTIC**" (GA-222927), the FP7-REGPOT-2008-1 project*

"***EUROSTRUCT***" *(GA-230146) and the FP7-REGPOT-2009-1 Project "**ARCADE**" (GA-245866)". Work at the Synchrotron Radiation Sources, **MAX-lab**, Lund, Sweden and **EMBL Hamburg Outstation**, Germany, was supported by the European Commission for the FP7 Research Infrastructure Action "**ELISA**" (European Light Sources Activities).*

#P50. High redundancy sensor system mimicking the olfactory epithelium

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The last 15 years have been witness to a great progress in understanding both the anatomical structure and the signal processing of the olfactory system. This has enhanced the research on artificial olfactory devices able to reproduce not only the structure of the biological system, but even its great performance, obtained thanks to a high degree of redundancy of the olfactory receptor neurons and the synergistic collaboration of its components. Following this tendency, we developed a large polymeric sensor array.

Conducting polymer technology was selected, because it is possible to tune the sensitivity of the polymers to make available many materials showing broad and overlapping specificity. Furthermore, the polymeric sensors feature low power consumption and rapid and reversible responses. Thanks to these characteristics, we implemented a high redundancy level in a versatile, low power and portable system, composed of 16536 sensors with 31 different types of material.

Initially, the device was calibrated generating different concentrations of Ethanol and Butanone. Then, the odour segmentation capability of the system was verified on mixtures of the two analytes, produced by decreasing the concentration of EtOH and increasing, proportionally, that of Butanone. A good separation among mixtures was achieved and the information on the analyte's concentration and identity was easily discriminated.

Finally, an experiment testing the system ability to distinguish different levels of an odour, butanone, in an environment with a fixed concentration of a second odour, EtOH, was executed. The results show that it is possible to resolve one component against a background of the other chemical.

#P51. Reliability tests performed on OFET Biosensors

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The development of fast and cheap biosensors as analytical tools is rising a great deal of interest in several research groups. These new analytical tools can be the alternative for the traditional biological assay largely performed all around the world.

Even thought traditional methods, like polymerase chain reaction (PCR) formats or the enzyme-linked immunosorbent assays (ELISA) (Bailey et al 2002), are high-throughput and reliable methods, they require sample pre-treatment like incubation steps, sample clean up, "labelling" the bio-analyte or amplification.

A viable alternative could be seen in the use of Organic Field Effect Transistors (OTFTs) as transducing tools for biosensor applications.

OFET biosensors have shown, the potential to offer very high performance level (Torsi et al 2008), while organic electronics allow to fabricate sensing circuits, also in an array configuration (Someya et al 2004), on flexible, paper substrates by low cost printing procedures.

Advantages of OTFTs may also reside in the chance of avoiding, or at least minimizing the sample pre-treating, fabrication costs, and to open up new perspectives in the development of portable and disposable device for a large area of applications, like medicine, food packaging, environment and many other.

We developed and tested a bio-OFET implementing phospholipids bilayers onto the device. Preliminary reliability data for the bio-OFET will be presented, along with a comparison of different deposition techniques for the phospholipids film. Morphological and structural investigations will be also discussed.

Future efforts will need to be focused on the development of new hybrid biomaterials integrated into electronic devices capable of being processed on flexible substrates.

Bailey et al (2002) FEMS Bicrobiol. Lett, 207, 153; L. Torsi et al (2008) *Nat. Mater*, 7, 412; T. Someya et al (2004) PNAS 101, 9966.

This work has been supported by the European Project "Gas Sensors on Flexible Substrates for Wireless Applications - FlexSMELL" Marie Curie International Training Network FP7-PEOPLE-ITN-2008.

#P52. The effective data compression using fuzzy c-means clustering and DWT at a very large gas sensor array system for mimicking biological olfaction

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It was reported that the latest sensor technology allows a 65536 element conducting polymer sensor array to be made with broad but overlapping selectivity to different families of chemicals emulating the characteristics found in biological olfaction.

However, the supernumerary redundancy always accompanies great error and risk as well as an inordinate amount of computation time and local minima at signal processing, like neural networks.

We propose a selection method for sensors using PCA and FCM algorithm to reduce the number of sensors for analysis by reducing redundancy between sensors. And we propose discrete wavelet transform for data compression in time domain.

The proposed method is as follows:

After reducing noise using a 3th butterworth low pass filter, four data points (one at transient state, two at steady state and one at recovery state) have to be selected for each sensor for feature extraction. Supposing the whole feature matrix has just one cluster, find the cluster center and membership grade of each sensor. By using membership grades, we can find the valid sensors without redundancy far away from the center. The rest of sensors except the valid sensors have much redundancy. For the rest of the sensor data, we can select cluster centers as you want. A nearest sensor around each cluster center is assigned to the valid sensors. The valid sensors can be used for further analysis.

The proposed method to compress data in time domain is as follows:

The valid sensors are compressed to 1/64 size in time domain using DWT.

To prove the proposed method, we have done a comparative analysis between gas sensor data with a reduced redundancy and without the reduced redundancy and reached a satisfactory result.

This work was supported by the National Research Foundation of Korea Grant funded by the Korean Government (NRF-2011-013-D00039)

#P53. Biomolecules integration in OTFT devices for biosensors development

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Organic Thin Film Transistors (OTFT) based bio-sensors are widely being studied because of their suitability for cost-effective mass fabrication on flexible substrates. The sensitivity and selectivity of OTFT based biosensors can be increased by integrating specific bio-receptor selective to the analyte molecules which are to be detected. Although many methods for bio-molecules immobilization exist, integrating bio-receptors on the active area of OTFT is a major challenge. Plasma Enhanced- Chemical Vapor Deposition (PE-CVD) of functional group to enhance the immobilization of bio-receptor on polymer substrate without affecting the bulk properties of material is one of the approaches. Novel method for integrating bio-molecules on the surface of poly(3-hexylthiophene) (P3HT) organic semiconductor using PE-CVD has been proposed. P3HT surfaces were plasma processed with- vapours of a mixture of acrylic acid and ethylene giving plasma deposited Ethylene/Acrylic acid (pdEthAA) coatings characterized at their surface by –(COOH/R) groups. Different plasma deposition times were evaluated to optimize the deposition period giving optimum electrical performance of the OTFT. The surface chemical composition of P3HT before and after PE-CVD was measured by X-ray photoelectron spectroscopy (XPS). The XPS data revealed presence of –COOH/R functionality on the plasma treated P3HT surface even few weeks after plasma deposition. The effect of annealing on electrical performance of the OTFT before and after PE-CVD was also investigated. The –COOH/R functionalized surface can further serve as an anchor for integrating the bio-receptors in the OTFT biosensors. (Torsi et al, 2005; Khan et al, 2010; Pistillo et al, 2005; Mourtas et al, 2011)

Torsi et al (2005) Anal. Chem., 70, 381A–387A.

Khan et al (2010) Adv. Mater., XX, 1–5.

Pistillo et al (2011) Surface and Coatings Technology, 205, S534–S536.

Mourtas et al (2011) Colloids and Surfaces B: Biointerfaces, 84,214-220.

This work is supported by Marie Curie FP7 PITN-GA-2009-238454 grant for European 'FlexSMELL' project "Gas sensors on flexible substrate for wireless applications".

#P54. A piezoelectric biosensor based on Odorant binding proteins

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We have developed olfactory sensors based on Odorant binding proteins (OBPs) to detect the presence of specific volatile organic compounds in the environment. OBPs are a sub-class of lipocalins, defined by their property of reversibly binding volatile compounds. They are ubiquitous proteins present in nasal mucosae of vertebrates, or in the sensillar lymph of insects at very high concentrations. They are able to bind a wide range of hydrophobic molecules, including odorants and are suggested to play an important role in perception of chemical signals. In this study four odorant binding proteins isolated from different species were tested. Two mutant OBPs from pig: OBPm2 and OBP-C an OBP of the European paper wasp (*Polistes dominula*) and a MUP (major urinary protein) from the mouse ere tested.

OBPs were coated using self assembled monolayers of thioctic acid or by direct adsorption on the gold surface of the transducer. Quartz crystals microbalances (QCMs) were employed as transducers to investigate the selectivity and the sensitivity of the biosensor to several pyrazines and polycyclic hydrocarbons, such as geosmin, in the vapour phase. Reproducibility and stability in the time were tested. The results showed the potential of OBPs to detect low concentration of analytes, in the order of *part per billion* for the high affinity compounds. Each OBP is able to discriminate the analytes tested. The signals measured are dependent on the strength of binding between the protein and the analytes, and are shown to be reproducible.

#P55. Comparative studies of the effects of exposure to an unpleasant and pleasant odorant in the olfactory system

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A number of reports suggest that sensory stimulation is important for the survival of olfactory sensory neurons (OSNs), however the effects of unpleasant odorant in the OSNs has not been studied well. Here we have investigated effects of either pleasant or unpleasant odorant stimulation in the olfactory system. First, we examined effects of odorant stimulation to animals in general. To this end, the behavior and body stress were monitored. 7 weeks old male mice showed evasion in unpleasant odorant (2-mercaptoethanol, 2-ME, stink) and exposure to stink increased the expression of corticotrophin-releasing-hormone (CRH) in the hypothalamus in 2 hrs and blood cortisol level in 6 hrs. In contrast, exposure to pleasant odorants (fragrance) did not show such increase. Next, we examined the cell viability in the olfactory epithelium (OE) upon odorant stimulation. Exposure to stink increased the apoptotic cells in the OE in 6 hrs. We also examined signal transduction pathways involved in odorant stimulation. Fragrance induced cAMP, but of our great interest stink did not induce cAMP. Moreover both of them activated MAPK pathways, but only stink induced cell death both in vivo and in vitro. In summary, our studies provide new intriguing aspects of olfactory function.

This work was supported by the DGIST R&D Program of the Ministry of Education, Science and Technology of Korea(11-BD-0401)

#P56. Smell the colour of the rainbow

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This paper explores a new 21st century body media multi-sensory 'rainbow', introducing computerized scent-output systems worn on the body for fashion, wellbeing and health applications. The rainbow merges technology and fashion with natural fragrances using the therapeutic power of essential oils to reflect the colours of the rainbow. The aim is to enhance mood and improve lifestyle, and will be placed alongside those in vogue such as alternative healing practices. It offers a fully personalized, controllable '*scent bubble*' experience which is intimate in nature and activated by the user alone from a collection of high-tech jewellery and clothing.

Designed for psychological end benefits and as a luxury fashion item, this collection can be programmed to deliver a palette of fragrances, depending on emotion, mood, occasion and time of day. Building on earlier work on 'eScent®' (Tillotson et al, 2006), minute delivery mechanisms can produce a selection of aromatic molecules in controlled ways, responding to personal needs. If combined with biometric sensors that measure stress indicators, soothing scents could be released whenever the stress levels exceed a certain threshold. Similarly, refreshing and revitalising scents can be used to fight fatigue and boost self-esteem. The ultimate goal is to embed electronic nose sensors within the structure of clothing to sniff stress and diseases.

Using colour in conjunction with therapeutic fragrances, gives the wearer a visual aid to alleviating their physiological and emotional state. State of the art technology, 21st century design features and the healing powers of nature combine to produce a fundamentally simple concept of creating a positive personal space on this highly charged 21st century planet we all share.

Tillotson.J., Manz.A., Jenkins.G, (2006),Proc IET Seminar on MEMS & Actuators, 2006 April

This study was supported by an Arts & Humanities Research Council (RCUK) Knowledge Transfer Fellowship in collaboration with Northumbria University and Philips Research.

#P57. Experience-dependent plasticity of the peripheral olfactory code in *Drosophila melanogaster* larvae

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During their larval stage, fruit flies are exposed to an odour-rich environment, in which they must choose between toxic and edible substrates. For this they need an efficient olfactory system with the capacity for both short and long term plasticity based on experience. Drosophila larvae possess only 21 paired olfactory sensory neurons (OSN), most of which express only one olfactory receptor (OR) and the co-receptor Orco. Information arising from each OSN is transmitted to a unique glomerulus in the antennal lobe and then to the mushroom body via projection neurons. Combinatorial coding in the periphery allows larvae to detect and discriminate a large number of odours.

We have exploited the UAS-Gal4 system, to create single Or lines in which only one, identified OSN is functioning. Using electrophysiology we have characterised the odour-response profiles of 19 of the 21 OSNs and found that an OSN's response to a given odour is often highly variable (Hoare et al 2008, 2011). This raised the possibility that the peripheral olfactory code exhibits plasticity and may therefore be involved in mediating behavioural adaptation and learning. In this study we will characterise neural correlates of behavioural conditioning at the level of the peripheral olfactory code. The aim is to generate a model of the peripheral code which can explain some of the components of experience-dependent changes in behaviour.

Hoare et al (2008) J. Neurosci, 28,9710–9722.
Hoare et al. (2011) PlosOne, 6(8) :e22996

This study is supported by BBSRC.

#P58. Digital coding of odors for olfactory information transmission using emotional adjective factors

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Usually odors have complex chemical compositions, it is difficult to precisely represent and identify as simple digital codes for olfactory information transmission regarding to multimedia application. It may be more effective to present odor using emotions of human being, who associates a smell in combination with feeling, rather than chemical analysis. If there are about 40-50 adjective factors describing odors perceivable to human beings, a number of odors can be expressed in adjective factors. In this study, we propose a technique of converting an odor into digital coding and decoding (restoring) for olfactory information transmission based on emotional adjective factors. Digital coding using odor expression factors involves converting an image of the odorous object into emotional expression factors having integer or real numerical data. Although more than 40 expression factors are chosen, only a few expression factors are practically involved in expressing a particular image of an odorous object, and the length of codes expressing image is not long. As a result, a small amount of memory has been used for representative odors and 95% or more of original data can be preserved. The coded odors are constructed as meta-data images and can be effectively reproduced after transmission. Thi primary result may be contributed olfactory information transmission and real communication applicable to various applications.

This study was supported by a research grant from ETRI (Electronics Telecommunication Research Institute) in Korea.

#P59. Relationship between odor qualities and molecular structures

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Odor character descriptors are used to represent the odor quality of substances and give us their semantic odor profiles. In our previous study, we analyzed odor descriptors to reveal the underlying dimensions for a great number of flavors using the reference by Arctander (1969)[1], which is one of the largest data banks of semantic odor profiles. Firstly, we classified the descriptors into 8 clusters which were named: ''Acid'', ''Sulfur'', ''Green'', ''Fruity'', ''Spicy'', ''Floral'' ''Woody'' and ''Phenolic''. Next, we examined common features of chemical structure for each cluster. Acid, Sulfur, and Phenolic clusters had their own features obviously in terms of functional group. However, the other 5 clusters had no clear feature, for instance, every 5 cluster had ester group compounds. Nevertheless,

distinctive features were found in each 5 cluster in terms of carbon skeletons. In this study, we conducted a human sensory test to compare the previous results obtained from analyzing the odor descriptors from Arctander's data and to reveal the relationship among 5 clusters in odor quality. These findings are of some help in understanding the relationship between odor qualities and molecular structures.

1 Arctander (1969) Perfume and flavor chemicals (aroma chemicals). Volumes 1 and 2. Allured Publishing Corporation, USA.

#P60. Building a robot maggot nose

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Robotic noses have the potential to be powerful diagnostic tools by detecting volatiles from pathogens in hospitals, monitoring air quality and by analysing sewage for water purity. Currently robotic noses are either single use diagnostic assays or expensive large arrays of sensors with high latency and high computational demand.

The peripheral olfactory system of *Drosophila melanogaster* larvae comprises 21 olfactory sensory neuron (OSN) types. The aim of this project is to understand how multiple odours are coded at this level. To address this, electrophysiological data was collected from a single OSN of larvae expressing a single olfactory receptor (OR74a), generated using the UAS-GAL4 system. Larvae were exposed to 1 s presentations of each of 5 biologically significant odours for signs of adaptation to the odours with repeat presentations, and for the spatiotemporal response to the odours.

Adaptation to repeat presentations of odours was not observed using a 20 s interstimulus interval. Early indications suggest that a single OSN can encode a different spatiotemporal profile of spikes for each odour. All presentations were statistically significant during the stimulation window. In addition, an existing odour delivery system was modified to facilitate increased data yield. A full profile of responses to odours at varying concentrations will be collected for OR74a and two additional lines, OR30a and OR24a. These data will then be used to construct a computational model of the peripheral olfactory system which considers the combinatorial code from OSNs to inform the design of a robotic nose.

This study was supported by the BBSRC.

#P61. Nutrient sensing receptors in gastric endocrine cells

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Sensing of protein in the luminal content is of particular importance for the regulation of digestive activities in the stomach, including the release of gastrin, the central hormone for controlling gastric activities. The molecular basis for tuning the release of hormones according to the protein content in the gastric lumen is still elusive. We have analysed the murine stomach for candidate amino acid receptors and concentrated on the broadly tuned amino acid receptor GPRC6A as a promising candidate. RT-PCR analyses of different stomach compartments provided evidence that GPRC6A is expressed in the stomach; especially in the gastric antrum. Using immunohistochemical approaches a large population of GPRC6A-positive cells was visualized in the basal half of the antral gastric mucosa. Molecular phenotyping of GPRC6A-immunoreactive cells revealed, that most of them contained the peptide hormone gastrin. A small population turned out to be immunoreactive for somatostatin. In search for additional amino acid receptors in the antral mucosa, we have found that also the calcium-sensing receptor CaSR is expressed in the antral stomach mucosa. CaSR-immunoreactivity was visible in many cells in the gastric antrum and it turned out that most of them contained gastrin; similar to GPRC6A cells. The finding that GPRC6A- and CaSR-receptors are both expressed in many if not all gastrin cells strongly suggests that both receptor types are co-expressed in the same cells, where they may contribute to the sensory capability of these cells to recognize protein breakdown products.

This work was supported by the Deutsche Forschungsgemeinschaft, BR 712/25-1. D. Haid gratefully acknowledges the receipt of a travel grant from ECRO.