24th international meeting

European Chemoreception Research Organization

Dijon | France

10|14 September 2014

https://colloque.inra.fr/ecro2014

ecro DIJON 2014

Contents

Welcome to the 24th European Chemoreception Research Organization Congress

On behalf of ECRO I would like to welcome all participants to the XXIV ECRO Congress 2014, in Dijon.

Our annual ECRO Congress is an important appointment to meet and discuss topics about chemosensory science. Plenary lectures and several Symposia will cover topics from many areas of chemosensation both in invertebrate and in vertebrate systems. Several poster sessions will give the possibility to young scientists to meet other young as well as more senior colleagues in a friendly atmosphere. Social events will further promote interactions and discussions among all participants.

ECRO 2014 is hosted in Dijon by the Centre des Sciences du Goût et de l'Alimentation (CSGA). Many thanks to Luc Penicaud and colleagues who nicely combined scientific and social events: we are all ready to savour an excellent combination of exciting science and local French flavors.

Anna Menini

ECRO President

Welcome from CSGA

The staff members of Centre des Sciences du Goût et de l'Alimentation (CSGA; Centre for Taste and Feeding Behaviour) and I are pleased to welcome you to the twenty fourth edition of the International Conference of ECRO.

The general objective of the CSGA is to get a better understanding of physico-chemical, molecular, cellular, behavioural and psychological mechanisms underlying sensory perception of food. The studies range from the release of aromatic substances and sapid molecules from the food matrix to the psychology and behaviour of consumer, through the biological events of food perception and food intake. The different units possess worldwide acknowledged and complementary competences in key-thematic fields: chemical analysis of complexity (pheromones, odorant, sapid and trigeminal molecules from food origin); release of compounds from food matrix; analysis of sensorial, cognitive and behavioural phenomenon associated to the treatment of sensory information, mechanisms involved in chemical communication, role of the internal and external environment (metabolism, development, experience, culture et and society). Research on Olfaction, Taste and related Behaviours are indeed thematic fields developed in the Centre thanks to talented researchers and technicians.

I do hope in the name of the organizing committee you will enjoy both the scientific program as well as the many charms of Burgundy, its wines and restaurants, its history, its landscapes and the magnificence of its architecture.

Taste and appreciate both science and pleasures!

Dijon welcomes ECRO meeting in 2004, under the presidency of Benoist Schaal. Ten years after it was a good opportunity and naturally we applied to welcome it again. Fortunately the ECRO board decide to select our candidature.

So have a good time and let's hope we might be able to see you in Dijon in 2024!

Luc Pénicaud
Head of the CSGA
President of the Organizing committee.

Organizing Committee

- Loïc BRIAND *(CNRS – CSGA – INRA - Université de Bourgogne, Dijon, France)*
- Jean-François FERVEUR *(CSGA-CNRS-Université de Bourgogne, Dijon, France)*
- Xavier GROSMAITRE *(CNRS – INRA – Université de Bourgogne, Dijon, France)*
- Sylvie ISSANCHOU *(CNRS – CSGA – INRA - Université de Bourgogne, Dijon, France)*
- Anna MENINI *(Neurobiology Group, SISSA, International School for Advanced Studies, Trieste, Italy)*
- Luc PENICAUD *(CSGA – INRA – CNRS - University of Dijon, Dijon, France)*
- Krishna PERSAUD *(School of Chemical Engineering and Analytical Science, The University of Manchester, UK)*
- Benoist SCHAAL *(Developmental Ethology and Cognitive Psychology Group – CSGA – CNRS – INRA – Université de Bourgogne, Dijon, France)*
- Didier TROTIER *(Institut de Neurobiologie Alfred Fessard – CNRS, Dijon, France)*

Biographies of Plenary Speakers

Jane Hurst

Jane is the William Prescott Professor of Animal Science at the University of Liverpool (since 1998), where she heads the Mammalian Behaviour & Evolution research group based in the Institute of Integrative Biology and Veterinary School. Her PhD on behavioural ecology of house mice (University of Birmingham 1981-1984) led to a long-term interest in scent communication and in kin recognition, pursued through a series of three UK Research Council fellowships at the University of Nottingham (1985-1998). Jane's current main research interests are in the functions, mechanisms and evolution of mammalian scent communication; rodent behaviour, reproductive strategies and pest management; and animal welfare. She has also been President of the Association for the Study of Animal Behaviour (2010-2012) and has been providing UK funding agencies with strategic advice on animal welfare and behaviour research since 2000.

Giovanni Galizia

C. Giovanni Galizia is Professor for Neuroscience and Zoology at the University of Konstanz, Germany, since 2005, and Director of the Zukunftskolleg, an institution for fostering young researchers across disciplines in their thrive for excellence in independent research. Galizia was born in Italy, he has studied biology in Berlin, Germany, and did a PhD in Zoology in Cambridge, UK (1993). After postdoc positions in Tübingen (Germany) and Berlin (Germany), he was an independent research group leader at the Freie Universität, Berlin, and was appointed Associate Professor for Entomology in January 2003. His field of interest is the olfactory coding in insect antennal lobe. The primary olfactory neuropil, the antennal lobe in insects, is organized in glomeruli. The glomerular activity patterns are believed to represent the across-fibre pattern of the olfactory code. Using optical imaging techniques, he measures the glomerular response to odour stimulation in various insect species.

Kazushige Touhara

Kazushige Touhara was trained in Japan (University of Tokyo) and obtained his PhD at the State University of New York (1993). After a post doc position at Duke University Medical Center (Mentor RJ Lefkowitz), he got an independent position as Assistant Professor at the University of Tokyo (Dpt Neurochemistry), Kobe (Biosignal Research Center) and Tokyo (Dpt Integrated Biosciences) successively. He is since 2009 Professor at the University of Tokyo, Dpt of Applied Biological Chemistry. His research interests include elucidation of molecular mechanisms underlying odorant and pheromone perception in both vertebrates and invertebrates. To understand how the mouse olfactory system can discriminate thousands of odorants and how mice send socio-sexual information to other individuals by using specific pheromone molecules. His team have focused on structural and functional aspects of odorant and pheromone receptors expressed by olfactory and vomeronasal sensory neurons, respectively. He also investigates signal transduction mechanisms in the insect olfactory system.

Ivan de Araújo

A natural of São Paulo, Brazil, Ivan de Araújo performed post-graduate work at the Universities of Edinburgh and Oxford, where he studied human brain representations of taste-odor combinations, fat perception, and thirst. During his post-doctoral work at Duke University he studied the responses of neuronal populations to changes in physiological state in both rats and mice. He joined the Pierce Laboratories and Yale University in June 2007, where his group has been focusing on the control of brain reward circuitries by peripheral physiological signals. He currently is an Associate Professor of Psychiatry at Yale's School of Medicine, Associate Professor of Physiology at Yale's School of Arts and Sciences, and an Associate Fellow at The John B Pierce Laboratory.

Conference Sponsors

General Information

Posters

Two posters sessions are organized: session 1 on Thursday and session 2 on Friday. Delegates are kindly requested to install and leave their poster on their attributed place for each day.

Badges

All delegates and speakers are kindly requested to wear their name during all conference activities. Name badges can be picked up from the registration desk on your first arrival.

Wi-Fi Access

A Free Hot-Spot WiFi connection is accessible.

Certificate of Attendance

A certificate of attendance can be obtained on request from the registration desk.

Registration Desk

The registration desk will be open during all the conference. A tourist information will be provided in the attendee's bag.

On-site Payment

On-site payment will be possible on Wednesday, September 10th, only for guided visits (reserved to specifically registrered people).

Liability and Insurance

In registering for this conference, delegates agree that organizers do not assume any liability whatsoever. It is highly recommended that all participants carry their own travel and health insurance.

Car parking

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Conference Presentation Program

Wednesday, September 10th

14:00 - 14:30 **Opening ceremony**: Anna Menini, ECRO President and Luc Pénicaud, Congress Organizer

14:30 - 15:30 **Plenary Lecture 1**: Jane Hurst (United Kingdom): Assessing kinship through scent – the mouse model. (Chairperson: Anna Menini) *(PL1)*

15:30 – 16:00 **Coffee break**

16:00 - 18:00 **Plenary Symposium 1: Sexual behaviour under olfactory control: from invertebrates to human: Org. Matthieu Keller** *(PlS1)*

- Post-mating olfactory switch-off in a male moth: smell, sex, and stop! : Christophe Gadenne *(OP1)*
- A wide range of pheromone-stimulated sexual and reproductive behaviors in female mice depend on G protein Galphao : Pablo Chamero *(OP2)*
- Hypothalamic response to the chemo-signal androstadienone in gender dysphoric children and adolescents : Julie Bakker *(OP3)*

► The role of glucocorticoids in reception of sex pheromones in the house mouse: Tatiana Laktionova *(short presentation 1)*

► Odorant-degrading enzymes in drosophila melanogaster : Martine Maïbèche *(short presentation 2)*

19:00 - 20:00 **Welcome reception** (City Hall)

Thursday, September 11th

09:00 - 10:00 **Plenary Lecture 2:** Kazushige Touhara (Japan): Chemosensory signals, receptors and behaviour (Chairperson: Xavier Grosmaitre) *(PL2)*

10:00 - 10:30 **Coffee break**

10:30 - 12:30 **Plenary Symposium 2: OdorSpace: The Nature of Olfactory Perceptual Space: Org. Noam Sobel** *(PlS2)*

- Mapping stimulus space onto perceptual space : Andreas Keller *(OP4)*
- Olfactory spaces: relating perceptual qualities to physical attributes : Moustafa Bensafi *(OP5)*
- From mapping odors in people-space to mapping people in odor-space : Noam Sobel *(OP6)*

► Specificities of chemical senses among sensory modalities: Tatsu Kobayakawa *(short presentation 3)*

► The capacity of humans to perceive components in a configural senary mixture and its sub-mixtures: Sébastien Romagny *(short presentation 4)*

12:30 - 13:30 **ECRO General Assembly**

13:30 - 15:00 **Lunch**

15:00 - 16:00 **Posters**

16:00 - 18:00 **Parallel Symposium 1: The central-nervous processing of taste in humans: Org. Emilia Iannilli** *(PaS1)*

- Speed, decisions and coding aspects of feeding : Sidney Simon *(OP7)*
- Taste, olfactory and food texture reward processing in the brain and the control of appetite : Edward T Rolls *(OP8)*
- Lateralized processing of taste in the human brain: Emilia Iannilli *(OP9)*
- Central taste disorders in humans : Basile N Landis *(OP10)*

► Modulation of central olfactory response by metabolic status and BMI in human: Claire Murphy *(short presentation 5)*

Parallel Symposium 2: Modeling olfaction: from odorant receptors to the olfactory bulb: Org. Johannes Reisert and Anna Menini *(PaS2)*

- Linking chemical and physiological aspects of odor space : Michael Schmuker *(OP13)*
- A dynamical feedback model for adaptation in the olfactory transduction pathway : Anna Boccacio *(OP12)*
- A tale of odorant and receptor: finding the best conformation : Tatjana Abaffy *(OP13)*
- Signal transduction and amplification in the confined cilia of olfactory neurons : Jürgen Reingruber *(OP14)*

► Transformation from a temporal code to rate code: Rafi Haddad *(short presentation 6)*

► Molecular dynamics simulations reveal the active and inactive states of olfactory receptors: Claire A. de March *(short presentation 7)*

18:00 - 19:30 **Posters**

Friday, September 12th

08:30 – 09:30 **Plenary Lecture 3**: Giovanni Galizia (Germany): Odor coding, identification and evaluation in insect neural networks (Chairperson: Jean-François Ferveur) *(PL3)*

09:30 - 10:00 **Coffee break**

10:00 - 12:00 **Plenary Symposium 3**: **Insect-Food Pheromone Interaction: Org. Jean-François Ferveur and Teun Dekker** *(PlS3)*

- Something does not smell right : induced plant volatiles inhibit sensory input and behavioural output in insect herbivores : Teun Dekker *(OP15)*
- Sex pheromones and flower odours : how a male moth finds a female in a noisy environment : Sylvia Anton *(OP16)*
- A challenging task for a male noctuid moth : scenting the conspecific female sex pheromone in the background of plant volatiles : Elisa Badeke *(OP17)*

- Sex pheromone and food search in caterpillars : Emmanuelle Jacquin-Joly *(OP18)*
- Larval and adult pheromones modulate larval food choice in Drosophila : Jean-François Ferveur *(OP19)*
- Starvation modulates coding of sex and habitat chemosensory signals in Drosophila : Sébastien Lebreton *(OP20)*

12:00 - 13:30 **Lunch**

13:30 - 14:30 **Posters**

14:30 - 16:30 **Parallel Symposium 3**: **Modulation of olfactory sensitivity and related behaviors by metabolic status: Org. Christine Baly** *(PaS3)*

- Hyperlipidemic Diet Causes Loss of Olfactory Sensory Neurons, Reduces Olfactory Discrimination, and Disrupts Odor-Reversal Learning: Debra Ann Fadool *(OP21)*
- Diet-induced obesity impairs olfactory-driven behaviors concomitantly with alterations of cellular dynamics and homeostasis in olfactory tissues : Marie-Christine Lacroix *(OP22)*
- Modulation of olfactory responsiveness by endocrine signals : Jörg Strotmann *(OP23)*
- Imaging odor-evoked activity in the olfactory bulb of ob/ob mice using Manganese-Enhanced MRI : Hirac Gurden *(OP24)*

► Negative impact of high fructose diet on olfactory abilities : Sébastien Rivière *(short presentation 8)*

Parallel Symposium 4: **Taste receptors and diet in wild and domestic animals: Org. Eugeni Roura and Scott McGrane** *(PaS4)*

- Taste adaptation to diet in Carnivora : Gary Beauchamp and Peihua Jiang *(OP25)*
- Physiological relevance of the Taste1 Receptor (T1R) family in the domestic cat and dog : Scott McGrane and Andy Taylor *(OP26)*
- Taste receptor expression and function in omnivorous farm animals : Eugeni Roura *(OP27)*
- Relating taste receptor function to feeding ecology and evolution : a cautionary tale : John I. Glendinning *(OP28)*

► Tuning breadths and receptive ranges of avian bitter taste receptors: Maik Behrens *(short presentation 9)*

► Experience affects mouse sweet-taste phenotypic behavior: David A Blizard *(short presentation 10)*

16:30 - 18:00 **Posters**

Friday evening

Gala dinner (Cellier de Clairvaux)

Saturday, September 13th

09:00 - 11:30 **Parallel Symposium 5**: **Decoding and mapping underwater olfaction: recent progress in zebrafish and Xenopus: Org. Ivan Manzini and Oliver Braubach** *(PaS5)*

- Olfactory neural circuitry mediating reproductive behaviour in zebrafish: Yoshihiro Yoshihara *(OP29)*
- Birth and migration of sensory neurons in the adult zebrafish olfactory system : Stefan Fuss *(OP30)*
- Expression of ancestral amphibian v2rs are expressed in the main olfactory epithelium of aquatic tadpoles as well as amphibious adults : Sigrun Korsching *(OP31)*
- Sulfated steroids are chemosensory stimuli in both the main and accessory olfactory system of an Amphibian : Ivan Manzini *(OP32)*

► Taste aversion in zebrafish juveniles: Brigitte Boyer *(short presentation 11)*

Parallel Symposium 6: **Molecular recognition and signalling in food sensing GPCRs: Org. Masha Niv** *(PaS6)*

- L-amino acid sensing G protein-coupled receptors and their role in food intake sensing : Hans Bräuner *(OP33)*
- Developing ligands for GPR41 and GPR43; receptors for short chain fatty acids produced by bacterial fermentation : Graeme Milligan *(OP34)*
- Molecular recognition of GPCR tastants: bitterness and promiscuity : Masha Niv *(OP35)*
- Regulators of G Protein Signaling (RGS proteins) and tastant signal transduction : David Siderovski *(OP36)*

► Intricate or feasible ? – Readouts from a genuine type II like human taste cell expressing multiple TAS2Rs: Michael Krohn *(short presentation 12)*

► Recombinant expression of the N-terminal domain of human T1R2 taste receptor : interaction with brazzein, a sweet-tasting protein : Laffitte Anni *(short presentation 13)*

10:00 – 10:30 **Coffee break**

11:30 - 12:30 **Plenary Lecture 4**: Ivan De Araujo (USA): The physiology and neural circuitry of sweet taste reward (Chairperson: Luc Pénicaud) *(PL4)*

12:30 - 13:00 **Closing ceremony**

13:00 - 14:00 **Lunch**

Saturday afternoon

Social events: guided visit of Dijon (reserved to specifically registered people)

Abstracts of Plenary Lectures

Plenary Lecture 1

Assessing kindship through scent – the mouse model

Jane Hurst[1]

[1] *Mammalian Behaviour & Evolution Group, Institute of Integrative Biology, University of Liverpool, Leahurst Campus, CH64 7TE, UK*

The ability to recognize kin has important potential fitness benefits in a variety of social contexts including parent-offspring recognition, inbreeding avoidance and cooperative breeding. While animals may recognize familiar individuals encountered during early development, using prior association during a sensitive period as a proxy for relatedness, many animals are able to recognize relatives regardless of prior familiarity. This implies the use of genetic markers to assess kinship, by phenotype matching to a recognition template learned from self and/or from familiar relatives. Scent cues have been strongly implicated in the ability to recognize unfamiliar kin across a broad range of species, although identification of the specific genetic markers used to recognize kin has proven particularly difficult. This is because, necessarily, polymorphic kinship markers must correlate strongly with sharing across the rest of the genome in normal animals. The genetic control provided by inbred laboratory mice has played a key role in identifying MHC-associated odours as one candidate marker, while our work on wild house mice has identified major urinary proteins (MUPs) as another candidate marker. Here I will critically review evidence from the different mouse models and experiments that have been used to assess the genetic markers in scent and the recognition templates involved in kinship assessment. I will also present some of our recent findings from experiments using wild mice, looking at both inbreeding avoidance and cooperation between females.

Plenary Lecture 2

Chemosensory signals, receptors, and behavior

Kazushige Touhara[1]

[1] *Department of Applied Biological Chemistry, and JST ERATO Touhara Chemosensory Signal Project, Graduate School of Agricultural and Life Sciences, The University of Tokyo*

In terrestrial animals, a variety of social and sexual behaviors are regulated by chemosignals called pheromones that act via the olfactory or vomeronasal system. Mice utilize both volatile and non-volatile pheromones that are recognized by olfactory receptors (ORs) and vomeronasal receptors, which belong to the G protein-coupled receptor superfamily.

In contrast, insect chemosensory receptors are ligand-activated nonselective cation channels. In both vertebrates and invertebrates, more and more chemosensory receptors have been deorphanized. Until recently, however, little has been known about volatiles emitted from individual animals that act as ligands for ORs in natural environments. Activity-guided fractionation of exocrine gland extracts and subsequent chemical analysis resulted in identification of unsaturated aliphatic alcohol as a natural ligand for a mouse OR.

Recently, we discovered the mouse OR that specifically recognized muscone, a unique macrocyclic odor utilized as a chemosensory cue in dear musk, and also the human muscone receptor.

These studies pave the way to exploring the function of each OR in a physiological context and the molecular basis for complex chemical communication between animals. Considering the number of orphan chemosensory receptor genes, there exist more previously-unidentified signaling molecules that affect animal behaviors.

Plenary Lecture 3

Odor coding, identification and evaluation in insect neural networks

Giovanni Galizia[1]

[1] *University of Konstanz, Germany*
E-mail: galizia@uni-konstanz.de

Much progress has been made recently in understanding how neural networks accomplish olfactory coding. The time is ripe to pull these results into a proposed connectivity network. Here, I will propose a wiring diagram for the major steps from peripheral processing all the way to behavioral readout, using insects as models. The major players are the antennal lobe (first processing network), the mushroom bodies (most complex brain structure, crucial for learning) and the lateral protocerebrum (containing the premotor control areas). Processing steps include a sequence of: (1) lateral inhibition in the antennal lobe, (2) nonlinear synapses, (3) threshold-regulating gated spring network, (4) selective lateral inhibitory networks across glomeruli, (5) feed-forward inhibition to the lateral protocerebrum. These cover most of the experimental results from different research groups and model species (while ignoring other important processing steps, e.g. within the receptor neurons themselves). I propose that the main difference between mushroom bodies and lateral protocerebrum is not about learned vs. innate behavior. Rather, mushroom bodies perform odor identification, while the lateral protocerebrum performs odor evaluation (both learned and innate). I will discuss the concepts of labeled line and combinatorial coding and postulate that under restrictive experimental conditions, these networks lead to an apparent existence of "labeled line" coding for special odors. Modulatory and peptidergic networks are proposed as switches between different evaluating systems in the lateral protocerebrum.

Plenary Lecture 4

The physiology and neural circuitry of sweet taste reward

Ivan De Araujo[1]

A vast array of organisms are strongly and innately attracted to nutritive sugars. Sugars not only have a pleasurable taste (sweet), but are also an essential source of energy; it remains however uncertain whether these two related and yet dissociate properties are sensed by the same neurons within the brain's reward circuitry. Recent work has contributed to dissect the neurocircuitry of sweet taste reward by revealing that sugars' sweetness and energy are sensed by segregated pathways within the brain's reward circuitry. Importantly, only sugars' energy, not sweetness per se, robustly stimulates the reward pathway mediating the acquisition of long-term behavioral habits and compulsions.

Abstracts of Plenary Symposium 1

Wednesday, September 10th

16:00-18:00

Plenary Symposium 1

Sexual behavior under olfactory control: from invertebrates to human

Matthieu Keller[1]

[1] *Behavioral & Reproductive Physiology,*
UMR 7247 INRA/CNRS/University of Tours, France

E-mail: mkeller@tours.inra.fr

Chemosignals are probably the signals that play the most important role in the control of sexual behaviour across both vertebrates and invertebrates. In this symposium, we propose speakers that will provide a broad overview of the actions of chemosignals in the context of sexual behaviour and sexual interactions. These presentations will illustrate this action in a wide diversity of animal models, ranging from invertebrates to vertebrates, including human.

We have chosen 3 presentations dedicated to various aspects of this field and that will respectively illustrate the olfactory switch-off of male moth after mating. (C. Gadenne, FR); the control of pheromone-stimulated sexual and reproductive behaviors in mice by vomeronasal G protein G alphao; and finally the responses of gender dysphoric children and adolescents to sexual odors such as androstadienone (J. Bakker, Amsterdam, NL and Liège, BE).

Because the proposal covers both a range of species, and various approaches and techniques including electrophysiology, behaviour or fMRI, we hope that this symposium will stimulate interest from a wide variety of researchers in the ECRO community, investigating questions across levels of analysis.

We hope that the proposal will gather the attention of the programm committee for the 2014 ECRO meeting.

Post-mating olfactory switch-off in a male moth: Smell, Sex, and Stop!

Christophe Gadenne[1, 2], Sylvia Anton[1]

[1] *Laboratoire Récepteurs et Canaux Ioniques Membranaires (RCIM), Université d'Angers, UPRES-EA 2647 USC INRA 1330, France*

[2] *E-mail: christophe.gadenne@angers.inra.fr*

In the male moth, *Agrotis ipsilon* , mating induces a transient inhibition of behavioural and central nervous responses to sex pheromone. Newly mated males are not attracted to sex pheromone, and the sensitivity of their antennal lobe (AL) neurons is lower than in virgin males. This transient olfactory inhibition takes place very early after the onset of the 2 h-copulation (a few minutes) and lasts during the whole scotophase. This switch-off prevents males from re-mating unsuccessfully until they have refilled their sex glands with the proteins that are needed for the production of a spermatophore. The mating-dependent olfactory plasticity is restricted to pheromone perception: newly-mated males still respond to plant odours. Moreover, the sex pheromone becomes inhibitory by differential central processing: below a specific threshold, it is not detected within the AL; above this threshold, it becomes inhibitory, preventing newly-mated males from responding even to plant odours. To explain the mechanisms of this rapid and transient olfactory switch-off, we have studied the role of biogenic amines such as octopamine (OA) and serotonin (5HT), and of hormones such as 20-hydroxyecdysone (20E). Amine treatments did not restore the behavioural pheromone response of mated moths. Although AL neuron sensitivity increased in newly mated males after injection of OA or 5-HT, only OA treatment affected certain response characteristics of AL neurons in virgin males. Whereas all measured AL neuron response characteristics were different between virgin and newly mated males, amine treatment in newly mated males restored only the latency and spike frequency, but not the duration of excitatory and inhibitory phases, which were initially found in virgin males. This shows that OA and 5-HT are probably not involved in the post-mating inhibition of responses to sex pheromone in *A. ipsilon* males. We also analysed the possible involvement of 20E, which is mainly produced in the sex accessory glands, on the post-mating switch-off of *A. ipsilon* males. Finally we searched for a potential factor, present in the sex accessory glands, which could induce the observed post-mating olfactory switch-off.

Altogether these results show that *A. ipsilon* males undergo a kind of post-mating sexual abstinence, by switching off their pheromonal system until their reproductive organs are ready for a next female encounter.

A wide range of pheromone-stimulated sexual and reproductive behaviors in female mice depend on G protein Gαo

Livio Oboti[1], Anabel Pérez-Gómez[1], Matthieu Keller[2], Eric Jacobi[1], Lutz Birnbaumer[3], Trese Leinders-Zufall[1], Frank Zufall[1], Pablo Chamero[1]

[1]Department of Physiology, University of Saarland School of Medicine, 66424 Homburg, Germany

[2]Laboratoire de Physiologie de la Reproduction & des Comportments, UMR 7247 INRA-CNRS-Université de Tours, F-37380 Nouzilly, France

[3]Laboratory of Neurobiology, Division of Intramural Research, National Institutes of Health, Research Triangle Park, NC 27709, USA

Optimal reproductive fitness is essential for the biological success and survival of species. The vomeronasal organ (VNO) is strongly implicated in the display of sexual and reproductive behaviors in female mice, yet the roles that apical and basal vomeronasal neuron populations play in controlling these gender-specific behaviors remain largely unclear. To dissect the neural pathways underlying these functions, we genetically inactivated the basal VNO layer using conditional, cell-specific ablation of the G protein Gαo. Female mice mutant for Gαo show severe alterations in sexual and reproductive behaviors, timing of puberty onset, and estrous cycle. These mutant mice are insensitive to reproductive facilitation stimulated by male pheromones that accelerate puberty and induce ovulation. Gαo mutant females exhibit a striking reduction in sexual receptivity or lordosis behavior to males, but gender discrimination seems to be intact. These mice also show a loss in male scent preference that requires a learned association for volatile olfactory signals with other nonvolatile ownership signals that are contained in the high molecular weight fraction of male urine. Thus, Gαo impacts on both instinctive and learned social responses to pheromones. These results highlight that sensory neurons of the Gαo-expressing vomeronasal subsystem, together with the receptors they express and the molecular cues they detect, control a wide range of fundamental mating and reproductive behaviors in female mice.

Hypothalamic response to the chemo-signal androstadienone in gender dysphoric children and adolescents

Sarah M. Burke, Peggy T. Cohen-Kettenis, Dick J. Veltman, Daniel T. Klink, Julie Bakker[1]

[1] *GIGA Neuroscience, University of Liège, Belgium & Netherlands Institute of Neuroscience, Amsterdam, The Netherlands*

The odorous steroid androstadienone, a putative male chemo-signal, was previously reported to evoke sex differences in hypothalamic activation in adult heterosexual men and women. In order to investigate whether puberty modulated this sex difference in response to androstadienone we measured the hypothalamic responsiveness to this chemo-signal in 39 prepubertal and 41 adolescent boys and girls by means of functional magnetic resonance imaging. We then investigated whether 36 prepubertal children and 38 adolescents diagnosed with Gender Dysphoria (GD; DSM-5) exhibited sex-atypical (in accordance with their experienced gender), rather than sex-typical (in accordance with their natal sex) hypothalamic activations during olfactory stimulation with androstadienone. We found that the sex difference in responsiveness to androstadienone was already present in prepubertal control children and thus likely developed during early perinatal development instead of during sexual maturation. Adolescent girls and boys with GD both responded remarkably like their experienced gender, thus sex-atypical. In contrast, prepubertal girls with GD showed neither a typically male nor female hypothalamic activation pattern and prepubertal boys with GD had hypothalamic activations in response to androstadienone that were similar to control boys, thus sex-typical. We present here a unique data set of boys and girls diagnosed with GD at two different developmental stages, showing that these children possess certain sex-atypical functional brain characteristics and may have undergone atypical sexual differentiation of the brain.

The Role of Glucocorticoids in Reception of Sex Pheromones in the House Mouse

Tatiana Laktionova[1, 2], Ilya Kvasha[1], Anna Voznesenskaya[1], Vera Voznessenskaya[1]

[1] *A.N.Severtzov Institute of Ecology and Evolution, Leninski prospect 33, 119071 Moscow, Russia*
[2] *Email: tatita.laktionova@gmail.com*

Olfactory cues play an important role in regulation of complex forms of social behavior including sexual behavior in mammals. A number of studies demonstrated a direct involvement of accessory olfactory system in regulation of male sexual behavior in mammalian species. While the role of sex hormones in regulation of perception and analysis of chemical signals are studied very well, the role of stress hormones remains quite unclear. At the same time suppressive effect of stress on reproduction of mammals is a well-known issue whereas influence of stress on signal perception in vomeronasal system remains unknown. Our earlier studies showed the suppression of the response to receptive female chemical cues of vomeronasal receptor neurons in males under exposure to emotional or metabolic stress. In the current study we compared the effects of acute vs chronic stress on the reception of sex pheromones in male mice. Four basic experimental approaches were used: behavioral, hormonal, immunohistochemical and pharmacological. Extended exposure to emotional stress affected performance of males in standard odor preference test. Males did not prefer odor of estrus female vs diestrus female ($n=40$, $p \leq 0.01$) while in control group of animals we observed such a preference. Patterns of sexual behavior were decreased in standard pairing test ($t=60$ min., $n=8$, $p<0.05$). Number of Fos-positive cells in vomeronasal receptor epithelium of males in response to receptive female chemical signals was significantly reduced in case of acute stress exposure ($n=8$; $p<0.05$). Fos-IR was fully blocked in case of chronic stress exposure. In search of putative mechanism in the current study, we investigated the expression of steroid receptors (glucocorticoid, GCR, androgens,AR, and mineralocorticoid, MCR) in vomeronasal receptor epithelium. We detected a profound GR-immunoreactivity in vomeronasal receptor tissue of male mice but not AR-immunoreactivity or MR-immunoreactivity, whereas it was present in control tissue. Abundant expression of GCRs in vomeronasal receptor tissue suggests possible direct action of stress hormones on receptor cells. The data obtained indicate glucocorticoid involvement in female chemical cues perception in vomeronasal system. Using pharmacological, immunohistochemical and endocrinological approach we have determined plasma corticosterone concentration range sufficient to block males response to receptive female odor at the behavioral, hormonal and at the level of vomeronasal epithelium: 230-250ng/ml. Pharmacological analysis showed a lack of influence of indicated corticosterone levels on the olfactory memory retrieval processes, which points to peripheral mechanisms of the response suppression to receptive female odor in male mice.

Supported by RFBR 14-04-01150.

Odorant-Degrading Enzymes in Drosophila melanogaster

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Peripheral reception of odorants includes several steps, from the transport of the odorant molecules by odorant-binding proteins, the interaction with the olfactory receptors leading to the depolarization pathway and the possible inactivation by odorant-degrading enzymes (ODEs). ODEs belong to various families of detoxification enzymes, such as carboxylesterases (CCEs), cytochromes P450 (P450s), UDP-glycosyl-transferases (UGTs) or Glutathione S-transferases (GSTs), mostly studied for their involvement in xenobiotic metabolism. We have first established the antennal transcriptome of *Drosophila melanogaster* in order to identify the whole set of antennal detoxification enzymes from this model species. Analysis revealed a high number of antennal detoxification enzymes, with at least 25 CCEs, 56 P450s, 31 GSTs and 9 UGTs. Their expression patterns through the body were then analyzed and compared between male and female antennae. Finally, several genes that showed overexpression or selective expression in antennae compared to other tissues were studied in more details - as putative ODEs - using biochemical (production of recombinant enzymes and kinetics assays), physiological (single sensillum recordings) and behavioral approaches, to test if the corresponding enzymes could play a role in the dynamics of odorant response.

Abstracts of Plenary Symposium 2

Thursday, September 11th

10:30-12:30

Plenary Symposium 2

OdorSpace: The Nature of Olfactory Perceptual Space

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Olfaction research is at a paradoxical state: We know a lot about **how** the olfactory system solves the problem of olfaction, but we have rather poor understanding or formulation of **what** the problem is. That is, what are the borders of olfactory perception, and what are its dimensions within these borders? How do you measure an odor in a meaningful way, and how do you measure an olfactory percept? A better understanding of olfactory perceptual space is critical for a full understanding of the neurobiology of olfaction.

In this symposium we will hear about progress in this area from three scientists from three different countries, and from a world-leading master perfumer. Dialogue with a perfumer goes beyond curiosity alone: the science of visual and auditory perception gained significant insight from dialogue with artists of vision and audition, namely painters/photographers and musicians respectively. The artists of olfaction are master perfumers, and we scientists have a lot to gain from dialogue with them.

Mapping stimulus space onto perceptual space

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Olfactory stimulus space consists of mixtures of a very large number of diverse molecules that can be mixed in different combinations and ratios. Predicting how a given olfactory stimulus is perceived is difficult. Part of this difficulty is due to the large inter-individual variability in olfactory perception and part is due to the influence of previous experiences and associations on perceptual judgments. We have conducted a series of psychophysical experiments involving single odor molecules as well as complex mixtures to improve our understanding of the relation between physical properties of the stimulus and perception. The goal of these studies is to make testable, quantitative predictions about the perceived quality of novel stimuli.

Olfactory spaces: relating perceptual qualities to physical attributes

Moustafa Bensafi[1], Arnaud Fournel[1], Camille Ferdenzi[1], Caroline Sezille[1]

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CNRS, INSERM, Université Claude Bernard Lyon 1, France

An important issue in olfaction research is to relate brain activity to physico-chemistry of stimuli and perception. We present here a brain imaging study aimed at relating neural activity in human olfactory cortex and chemical space of odorants on the one hand and perceptual space of odors on the other hand. By combining Multi-Voxel Pattern Analysis and fMRI, we found distinct spatial activity patterns in piriform cortex (PC) as a function of chemical and perceptual similarity: whereas anterior PC activity significantly correlated with similarity in odorant physicochemical features, posterior PC activity significantly correlated with olfactory perceptual similarity (i.e. odor intensity, familiarity, pleasantness and edibility). Such effects were not observed in human amygdala. Rather, spatial activity of this area was significantly correlated with trigeminal perceptual similarity (i.e. irritation, coolness, warmth and pungency). Combined with previous works, these findings strengthen the notion of segregated neural representations for chemical space and perceptual space of odors.

From mapping odors in people-space to mapping people in odor-space

Noam Sobel[1]

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Exactly one hundred years ago, Alexander Graham Bell asked: “Can you measure the difference between one kind of smell and another? It is very obvious that we have very many different kinds of smells, all the way from the odor of violets and roses up to asafetida. But until you can measure their likenesses and differences you can have no science of odor”. Over the past several years our group has developed olfaction-relevant measures for the structure of odor molecules and for odor perception. The resultant olfactory metrics serve to predict a modest but significant portion of odorant induced perception and neural activity from odorant structure alone. This allowed empirically answering Bell's above question (in Angle Distance Metric): Rose to Violet = 0.3239, Rose to Asafetida = 0.3754, Violet to Asafetida = 0.3488. Here I will first briefly review these efforts, and then detail how our metric approach implies two novel olfactory phenomena: The first is an implied point of sensory convergence where all olfactory mixtures should smell the same. We call this point "olfactory white". The second is implied points beyond the upper and lower boundaries of our metric space, which should therefore be odorless. We call these points "infrasmell" and "ultrasmell". Finally, given a valid measure of odor perception, we can now reverse our model and numerically characterize an individual based on his/her unique olfactory makeup. This yielded what we call "olfactory fingerprints" that allow us to identify one out of four million individuals, and predict various non-olfactory traits such as HLA makeup.

Specificities of chemical senses among sensory modalities

Naomi Gotow[1], Tatsu Kobayakawa[1]

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Vision is physical sense, whereas olfaction and gustation are chemical senses. Active sensing might works in vision, olfaction, and gustation, whereas passive sensing might work in vision and olfaction except for gustation. The perceptual stability in everyday life, on the other hand, is supported by integrating multimodal information. In order to keep perceptual stability for environment that continues changing by various events, synchrony perception for cross-modal combinations would surely play an important role.

We examined whether each sensory property affected synchrony perception by performing temporal judgment for three cross-modal combinations using visual, olfactory, and gustatory stimuli (V-O, V-G and G-O). We prepared 31 stimulus onset asynchrony (SOA) conditions from −1900 to 1900 milliseconds between standard and comparison stimulus in three sensory combinations. And participant was asked whether two stimuli belonging to different modalities were presented simultaneously, or not. Ten persons were participated and 280 trials were presented for each modality combination. After calculating probability distribution of simultaneity judgments in each SOA, we calculated half width at half height (HWHH) and point of subjective simultaneity (PSS) on the basis of temporal distributions of simultaneous rates in each combination, and compared HWHH and PSS among three cross-modal combinations. HWHH demonstrates temporal resolution of related modalities.

Although HWHH did not differ among V-O, V-G and G-O combinations, HWHH exhibited higher value in cross-modal combinations involving one or two chemical stimuli than in combinations of both physical stimuli reported in the previous study. PSS of V-O combination was approximately equal to point of objective simultaneity (POS), whereas PSS of V-G, and G-O related to gustation, receded greatly from POS. In V-G, and G–O combinations, both PSS shifted in direction of visual or olfactory stimuli faster, distances between POS and PSS significantly differed between these two cross-modal combinations. We considered that prior entry effect was more strongly produced in olfactory–gustatory combination than in visual–gustatory combination. These results might express the strength of relationship between sensory modalities in everyday life.

The capacity of humans to perceive components in a configural senary mixture and its sub-mixtures

Sébastien Romagny[1], Gérard Coureaud[1], Thierry Thomas-Danguin[1]

[1] *Centre des Sciences du Goût et de l'Alimentation (CSGA), UMR 6265 CNRS, UMR 1324 INRA, Université de Bourgogne*

Humans have limited elemental abilities with regard to odor mixture analysis. As repeatedly shown by the group of David Laing, human adults can hardly identify all the odorants in a mixture composed of more than 4 odorants. This limit seems to be especially independent of the nature of the odorants included in the mixture, but also on their ability to blend within the mixture, i.e. whether the mixture is processed in an elemental or configural way. However, the identification procedure used in these studies involved a major role of odor memory or semantic processing. To overcome this issue, we set out a direct perceptual paradigm relaying on a paired comparison test to assess whether a human panel (n=22) can recognize odorants in a senary mixture perceived configurally. This 6-odorant blending mixture (RC) was previously shown to evoke spontaneously a red cordial odor, which is different from the odor quality of each of its single odorants. We also determine the identification ability of the panel for each RC sub-mixture with the hypothesis that single odorants would be more readily recognizable as compared to the whole mixture (configural processing). A 12-ways dynamic dilution olfactometer was used to deliver a pair of stimuli to compare. The first stimulus (target) was one single odorant from RC and the second stimulus (sample) was one single odorant, a sub-mixture of RC (of 2 to 5 odorants) or the RC mixture. Each participant had to indicate the presence (yes/no) of each target in all samples for a total of 378 pairs. The signal detection theory was applied to analyze the data and the discriminability index d' was used to reflect target recognition performances. The results revealed a high level of recognition for samples including single odorants (target vs. single odor). This performance dramatically decreased in binary sub-mixtures and mixtures containing 3 or 4 odorants. The recognition level was no more significant for sub-mixtures including 5 odorants. Moreover, we found significant differences of recognition performance depending on the target, i.e. on the nature of target. For instance, while vanillin was usually well identified even in 4-odorant sub-mixture, the identification rate of ethyl acetate was low even in binary mixtures. Taken together, these results confirm the limit of perceptual analysis of odor mixtures (4 odorants) but, in contrast to previous reports, suggest differences in the salience of some odorant which appear more readily recognizable in mixture.

Abstracts of Parallel Symposium 1

Thursday, September 11th

16:00-17:00

Parallel Symposium 1

The central-nervous processing of taste in humans

Emilia Iannilli[1]

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Eating is of central importance to our lives. This is why different fields, like medicine, psychology, sociology, but also the public exhibit a strong interest in “taste”. Understanding of the basic physiological mechanisms has become more and more complete; however, especially with regard to the central-nervous processing of taste, several aspects remain unclear. In this Symposium we aim to discuss recent results obtained with neuroimaging and electrophysiological research on human gustation. Specific highlights include laterality, taste-mechanosensory integration, age dependency of the taste perception as well as taste dysfunction and the new ways of its investigation.

Speed, decisions and coding aspects of feeding

Sidney Simon[1]

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Coding schemes throughout the gustatory system will be critically discussed. Information will include speed of perception, the value of using behaving animals and responses to the "primary" tastants and water. .

Taste, olfactory and food texture reward processing in the brain and the control of appetite

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Recordings of neuronal activity, and functional neuroimaging in humans, show that the primary taste cortex in the anterior insula provides separate and combined representations of the taste, temperature, and texture (including fat texture) of food in the mouth independently of hunger and thus of reward value and pleasantness. One synapse on, in the orbitofrontal cortex, these sensory inputs are for some neurons combined by learning with olfactory and visual inputs, and these neurons encode food reward in that they are modulated by satiety signals in that they only respond to food when hungry, implement sensory-specific satiety, and in that fMRI activations correlate with subjective pleasantness of flavor. Cognitive factors, including word-level descriptions, and attention, by a top-down process, modulate the representation of the olfactory and taste reward value of food in the orbitofrontal cortex.

Further, there are individual differences in the representation of the reward value of food in the orbitofrontal cortex, in that for example the orbitofrontal and anterior cingulate cortex reward systems respond more to the sight and flavour of chocolate in chocolate cravers than in non-cravers. There are also age differences in flavor processing, with for example the unpleasantness of tomato/vegetable juice in the young related to low activations in pleasantness-related regions of the amygdala and high activations in unpleasantness-related regions in the anterior cingulated cortex. .

Decisions about reward value are taken by a third cortical tier of processing in the ventromedial prefrontal cortex, area 10. .

Implications of this brain foundation of sensory processing, reward value, and decision-making will be discussed.

Lateralized processing of taste in the human brain

E. Iannilli [1,2]

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The focus of this talk is to elucidate recent advances in human gustatory pathways with respect to the laterality of the gustatory responses. Psychophysical and neuroimaging studies will be examined. 6 models and their experimental evidences will be discussed trying to highlight the common traceable clues.

Central taste disorders in humans

Basile N. Landis[1]

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The exact pathway of gustatory fibres has been a matter of debate for at least the last two hundred years. Important parts of the knowledge on human taste anatomy we know currently, is based on clinical observations done in patients with known and circumscribed lesions and consecutive symptoms or taste deficiencies. This is has been the case for the description of peripheral but also central human taste anatomy. It is only recently that functional imaging techniques further confirmed what had been assessed or postulated based on observations in patients with lesions.

The presentation gives a short historical and patient based overview about current knowledge in human taste anatomy and related peripheral and central gustatory impairments.

Modulation of Central Olfactory Response by Metabolic Status and BMI in Human

Claire Murphy[1], Aaron Jacobson[1], Lori Haase[1], Erin Green[1]

[1] *San Diego State University, the University of California, San Diego, SDSU/UCSD Joint Doctoral Program, San Diego, CA, USA*

Worldwide, the obesity epidemic is an increasing public health concern. Olfaction is a powerful driver of appetite and nutrient consumption. It's role in the development and maintenance of obesity is a matter of keen interest. Research in animal models suggests that metabolic state and obesity are associated with olfactory response (Palouzier-Paulignan et al., 2012). Hunger increases and satiety decreases odor detection in rodents. Rodents fed a high fat diet show odor seeking behavior that suggests modulation of the olfactory system by metabolic status and hormones involved in satiety. Differences in olfactory response in central brain regions have been observed electrophysiologically in rodents fed a high fat diet. We have reported differences in fMRI response to *taste* in hunger and satiety, that differed as a function of obesity (Green et al., 2011); however, there is a paucity of information available regarding whether central brain response to odor in humans is related to obesity and whether effects are seen in satiety as well as hunger. Reports of the effects of obesity on threshold sensitivity in humans are mixed. We investigated odor detection sensitivity in a life-span sample of 60 adults, half obese, half normal controls. BMI predicted human olfactory threshold sensitivity. In Zamora et al., (2012) we reported a positive linear relationship between adiposity (both BMI and waist circumference) and latency of the brain response to odor recorded with event related potentials in older adults. Here we report an fMRI study in which we investigated central response to a food odor in humans who were scanned in both fasted and sated states in separate, counterbalanced sessions. Functional imaging was conducted on a 3T GE Excite scanner using a standard gradient echo EPI pulse sequence to acquire T2*-weighted functional images. Citral was presented in the mouth in a paradigm that mimics natural flavor perception (See Haase et al., 2007 for stimulus delivery methods). Subjects rated the pleasantness of the odor while in the scanner using the gLMS. AFNI was used to conduct whole brain analysis and region of interest (ROI) analysis, the latter guided by the literature on olfaction, reward and feeding. Activation was associated with degree of adiposity. ROI analyses in olfactory and reward areas demonstrated differences as a function of BMI and hunger and satiety. Results suggest that brain activation during olfactory stimulation is associated with obesity and metabolic state in humans.

Supported by NIH grant #AG004085-26 to CM.

Abstracts of Parallel Symposium 2

Thursday, September 11th

17:00-18:00

Parallel Symposium 2

Modeling olfaction: from odorant receptors to the olfactory bulb

Johannes Reisert[1], Anna Menini[2]

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The four speakers, all in the early stages of their careers and from four different countries, will explore the recent and accelerating progress made in viewing olfactory processes in a highly quantitative light. Mathematical approaches have become increasingly feasible over the recent years aided by ever improving understanding of many aspects of olfaction and by improved experimental approaches used to address olfactory function. Tatjana Abaffy will explore how odorant receptors bind their ligands and how this might lead to agonistic or even antagonistic interaction. Anna Boccaccio will describe how understanding olfactory transduction mathematically helps to understand olfactory adaptation, while Juergen Reingruber will investigate the consequences of olfactory transduction occurring in the very small volume of olfactory cilia. Michael Schmuker concludes in investigating the chemical and physiological coding in the olfactory bulb.

We believe that this proposal will provide a broad and timely overview of olfactory function and as such will be interesting to a large range of ECRO participants. It will also provide the opportunity for four presenters, three from Europe, one from the USA and all in the early stages of their careers, to present their recent achievements. Drs. Abaffy, Boccaccio and Schmuker already have an impressive body of work within olfaction, while Dr. Reingruber has only recently began to work on olfaction, having previously focused on modelling photoreceptor transduction.

We hope you will find our proposal interesting and worthy to be included in ECRO 2014 and are looking forward to hearing from you.

Linking chemical and physiological aspects of odor space

M. Schmuker[1], Jan Sölter[2]

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The primary sensory areas in the visual, auditory and somatosensory systems exhibit a spatial organization that mimics the topography of the stimulus space along it's principal dimensions. In olfaction, a rigorous definition of such a relationship is still missing. Two major challenges to investigate this relationship exist: First, we need to know the molecular receptive ranges of as many glomeruli in the primary olfactory brain centers as possible. Second, for a thorough mathematical analysis of chemotopy we require a suitable numerical representation of odorants that captures essential properties of the olfactory chemical space.

To overcome the first challenge, we developed a method for automatic segmentation of glomeruli in intrinsic optical signal imaging in the olfactory bulb. Our method is based on non-negative matrix factorization and allows to incoporate prior knowledge about the temporal and spatial characteristics of glomerulus responses. Its segmentation performance compares favorably against established approaches based on independent component analysis both in terms of noise resilience and number of identified glomeruli. We applied this method to the analysis of a large set of *in-vivo* intrinsic optical signal recordings of glomerular responses to many odorants. The method enabled reliable identification of glomeruli across individuals based on their response spectrum. It thus largely alleviated the need for genetic markers linked to specific ORNs, making the aquisition of molecular receptive ranges easier.

To tackle the second challenge, we assessed the performance of a variety of molecular descriptor sets to predict odorant receptor responses in Drosophila. A 1500-dimensional physicochemical descriptor space delivered best prediction performance, but its high dimensionality made it difficult to handle. When considering lower-dimensional representations with around 30 features, descriptors that analyse molecular vibrational modes performed as well as a low-dimensional selection of physicochemical properties. This result suggests that a description of chemical space based on the flexibility of molecules is well suited to describe odorant space and points out a promising direction to develop better chemical descriptors for odor space.

We now combine these methods to explore chemotopy in the dorsal olfactory bulb of mice.

A dynamical feedback model for adaptation in the olfactory transduction pathway

Anna Boccaccio [1, 2], Giovanna De Palo [2], Andrew Miri [2], Anna Menini [2], and Claudio Altafini [2]

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Detection of odorants occurs in the olfactory sensory epithelium in the nasal cavity. Here in the ciliary membrane of vertebrate olfactory sensory neurons, odorant molecules bind to odorant receptor proteins triggering the olfactory transduction cascade that produces an electrical signal.

Adaptation to repetitive or maintained odorant stimuli starts at the level of olfactory sensory neurons through a Ca^{2+} -dependent modulation of the olfactory transduction cascade. Two distinct types of adaptation can be distinguished. We denote them respectively multipulse and step adaptation.

Adaptation to repetitive stimuli denoted here as multipulse adaptation, appears as a decrease in the amplitude of the second of two consecutive responses when the sensory neuron is stimulated with two brief identical odor pulses. Adaptation to a maintained stimulus, denoted as step adaptation, occurs in response to a sustained step-like odor stimulation and is characterized by a return to a steady state current amplitude close to the pre-stimulus value, after a transient peak.

In spite of the evidently similar nature of the two phenomena, mathematical models taking into account both forms of adaptation are very rare.

We propose a minimal dynamical model of the olfactory transduction pathway that reproduces both step and multipulse adaptation. The model includes the kinetics of the CNG channels, the concentration of Ca^{2+} flowing through them, and the Ca^{2+} -complexes responsible for the regulation.

We show that both forms of adaptation can be well described using different time constants for the kinetics of the Ca ions (faster) and the kinetics of the feedback mechanisms (slower).

The model is validated on experimental data collected in voltage clamp conditions using different techniques and animal species.

A tale of odorant and receptor: finding the best conformation

Tatjana Abaffy[1], Selvan Bavan, Benjamin Sherman, Charles W. Luetje

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Understanding the molecular receptive field of olfactory receptors and deciphering the olfactory recognition code are hampered by the huge number of odorants and large number of olfactory receptors, as well as the complexity of their combinatorial coding. Most olfactory receptors remain orphan receptors with unknown ligands, despite successful methods of cell based heterologous expression systems. Here, we present a novel strategy to deorphanize these receptors, an *in silico* high-throughput approach. A virtual library of 574 odorants was screened against a mouse olfactory receptor MOR42-3 and the top 40 candidate ligands were selected using two different scoring functions. These odorants were then tested *in vitro*using the *Xenopus* oocyte heterologous expression system and two-electrode voltage clamp electrophysiology. The candidate ligands were screened for both agonist and antagonist activity. Nineteen of the compounds were validated as agonists, while 3 were found to be antagonists. The high positive predictive value of our *in silico* approach is promising. We believe that this approach can be used for initial deorphanization of olfactory receptors as well as for future comprehensive studies of molecular receptive field of olfactory receptors.

Signal transduction and amplification in the confined cilia of olfactory neurons

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The sensory units of an olfactory receptor neuron (ORN) are long and slender cilia, where odorant recognition and transduction takes place. Contrary to phototransduction, the detection of odorant molecules does not depend foremost on a biochemical protein amplification cascade. Instead, odorant exposure leads first to the opening of Ca^{2+}-permeable cyclic nucleotide-gated (CNG) channels. The subsequent Ca^{2+} influx leads to the opening of Ca^{2+} activated Ano2 chloride channels that generates a large chloride current which is the major amplification step. In the small ciliary volume of around 1 femtoliter the exchange of a single ion already leads to a concentration change around 2nM. Thus, currents can rapidly shift reversal potentials and change the electrical driving forces. How does this affect the olfactory response? Why is the current carried first by cations and then anions? What is the role of the chloride current?

To study such questions we developed an electrodiffusion model that accounts for the biochemical transduction pathway and the complex ion dynamics in the confined cilium geometry. This model allows studying the olfactory response in great detail by dissecting the contributions of the various ionic fluxes and the biochemical reactions. Simulations reveal complex ion dynamics in the cilia. For example, the current fraction carried by various ions is not the same for the currents between cilia and mucus compared to cilia and cell body. Furthermore, the simulations suggest that the characteristic initial peak of the odorant response is not generated by the transduction pathway, but is an electrical property due to the ion dynamics.

Transformation from a temporal code to rate code

Nashige Uchida[1], Rafi Haddad[2]

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[2] *The Gonda Multidisplinary Brain Research Center, Ramat-Gan, Israel*

Odor stimulation evokes complex spatiotemporal activity in the olfactory bulb, suggesting that the identity of activated neurons as well as the timing of their activity convey information about odors. However, whether and how downstream neurons decipher these temporal patterns remains debated. We addressed this question by measuring the spiking activity of downstream neurons while optogenetically stimulating two foci in the olfactory bulb with varying relative timing in mice. We found that the overall spike rates of piriform cortex neurons were sensitive to the relative timing of activation. Posterior piriform cortex neurons showed higher sensitivity to relative input times than neurons in the anterior piriform cortex. In contrast, olfactory bulb neurons rarely showed such sensitivity. Thus, the brain can transform a relative time code in the periphery into a firing-rate-based representation in central brain areas, providing evidence for the relevance of relative time-based code in the olfactory bulb.

Molecular Dynamics simulations reveal the active and inactive states of Olfactory Receptors

Claire A. de March[1], Nicolas Baldovini[1], Anne-Marie Le bon[2], Yiqun Yu[3], Kaylin Adipietro[4], Minghong Ma[3], Hiroaki Matsunami[4], Jérôme Golebiowski[1]

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[4] *Duke University Medical Center, 261 CARL Building, Durham, NC 27710, USA*

The complexity of the odor chemical space and the large number of ORs associated to their combinatorial activation make understanding odor coding an enormous challenge. More specifically, being able to predict the behavior of an olfactory receptor in front of an agonist, an antagonist or a non-agonist remains to be done.
Using a joint approach combining molecular modeling and experimental data on several ORs, we have built a model that can capture the active or inactive state of these proteins when bound to ligands with different potencies.
The methodology is illustrated on few challenging cases. We predict the affinity of various sandalwood odorants for the human OR1G1 receptor, the activation mechanism of OR7D4 by its strong agonist, androstenone and androstadienone, and the response of cells expressing the mouse olfactory receptor mOR256-3.
Such powerful approaches will help unravel odor-coding in the nervous system and facilitate the understanding of general rules of odor-induced activation of the olfactory neurons in the nose.

Claire A. de March thanks Giract for a PhD bursary and the Roudnitska foundation for funding her PhD thesis. The Olfactome project is funded by the région Provence Alpes Côte d'Azur.

Abstracts of Plenary Symposium 3

Friday, September 12th

10:00-12:00

Plenary Symposium 3

Insect-Food Pheromone Interaction

Jean-François Ferveur[1], Teun Dekker[1]

[1] *CSGA-CNRS-Université de Bourgogne, Dijon, France*

In nature, most animals use odorants cues to locate mates and food sources. In insects, food and pheromone can interact at multiple levels. Food odors may synergize attraction to pheromones, and vice versa. Adult and larval pheromone can modulate attraction and food choice in juvenile insects. Pheromones can be derived from the mutualist action of microorganisms on the food ingested. Food and pheromonal molecules can also interact at the molecular levels when they are perceived both by the olfactory and taste sensory systems. The temporal food-pheromone association experienced during early development may also change mate and/or food choice. Interactions can take place at various levels of organization and in different contexts. These findings are particularly relevant with respect to the "plant-insect" and "host-parasitoid" more or less specialized association. Rapid progress in this field comes from the convergence of powerful techniques from different areas of science including chemical ecology, chemistry, animal behavior, genomics and molecular biology. This is especially true for the model organism *Drosophila melanogaster* and moth species, which allow us to utilize a unique combination of techniques. The fast development of the field and tools, however, permit addressing questions also in an increasing number of organisms.

The 6 speakers gathered for this symposium will present "state of the art" tools and concepts used to decrypt, at these multiple levels, food-pheromone interaction. Their presentation will focus on investigations carried on (*i*) neural structures (sensory systems and brain centers), (*ii*) behavior of juvenile and adult individuals, and (*iii*) genetic and environmental mechanisms underlying the evolution of food-pheromone perception. All talks aim to converge to provide an integrative view on chemo-induced behaviors with a particular focus on feeding behavior and mate choice.

Something does not smell right: induced plant volatiles inhibit sensory input and behavioural output in insect herbivores

Eduardo Hatano[1,2], Saveer M. Ahmed[1], Felipe Borrero-Echeverry[1], Martin Strauch[3], Muhammad Zakir Ali[1], Marie Bengtsson[1], Bill Hansson[4], Rickard Ignell[1], Peter Anderson[1], Paul Becher[1], Peter Witzgall[1], Teun Dekker[1]

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Plants under herbivore attack release volatile signals that attract parasitoids. Herbivores in turn avoid such plants, because of the higher risk of parasitization. Which volatiles herbivores use, and how deterrence is coded, are unknown. Here we demonstrate that in *Spodoptera littoralis* induced cotton volatiles suppressed orientation to host plants as well as to mates perched on these. We then found a surprising convergence. The homoterpene, (E)-4,8-dimethyl-1,3,7-nonatriene (DMNT), an induced volatile used by parasitoids to find hosts, is also key in herbivore deterrence: addition of DMNT to a synthetic cotton mimic suppressed plant odour and pheromone orientation. Using calcium imaging in the antennal lobes, we dissected the neurophysiological basis of this interaction. This revealed that DMNT, at concentrations that did not induce a measurable neurophysiological response alone, attenuated responses to both pheromone and (Z)-3-hexenyl acetate, a known host-plant attractant. Subsequent sensory neuron recordings showed that the suppression of the response to pheromone is peripherally mediated. Apparently, olfactory sensory inhibition, which has previously been reported without reference to an animal's ecology, can be a core part of coding of ecologically relevant odours. As DMNT, and possibly other related synthetic compounds, attract parasitoids and deters herbivores, they may be useful in the development or enhancement of push-pull strategies for sustainable agriculture.

Sex pheromones and flower odours: how a male moth finds a female in a noisy environment

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Moths, like many other insects, communicate using olfactory cues. Females produce a species-specific sex pheromone, which is detected by males over large distances. Male moths also use flower odours to find food or may use host plant odours as additional cues to localize females. Plant-derived odours, present in large amounts in a natural environment, may, however, also represent an olfactory noise, possibly disturbing specific communication.

In the noctuid moth *Agrotis ipsilon* , virgin male attraction to the sex pheromone increases when presented together with a flower odour. However, different plant compounds added to the pheromone during orientation can have different behavioural effects in this species. To elucidate the neuronal mechanisms underlying behavioural effects, we investigated peripheral and central nervous processing of pheromone-plant odour mixtures applied simultaneously or as a background stimulus. Heptanal, a major component of linden flowers, elicited unexpectedly excitatory responses in pheromone-specific olfactory receptor neurons and inhibited responses when added to the pheromone. Extracellular single sensillum recordings revealed that pheromone responses in these olfactory receptor neurons were decreased when applied in a background of heptanal. Pheromone responses in the male-specific part of the antennal lobe, the macroglomerular complex (MGC) were also reduced when applied together with heptanal during optical imaging recordings. Intracellular and extracellular MGC-recordings revealed a reduced response to the pheromone-heptanal mixture as compared to the pheromone alone in a majority of olfactory neurons. These neurons improved their capacity to follow stimulus pulses upon mixture stimulation.

In the plant odour specific part of the olfactory system, no interaction of the sex pheromone with plant odours was found at the antennal level. Plant-specific olfactory receptor neurons did not respond to the pheromone and the pheromone did not modify plant odour responses. This lack of a mixture interaction persisted up to the antennal lobe input level. Calcium responses within the so-called ordinary glomeruli, serving plant odour processing, were not modified by the pheromone. A large proportion of antennal lobe neurons with dendritic arborisations in the ordinary glomeruli, however, responded more strongly to the mixture of heptanal and pheromone than to the pheromone alone, even though only weak responses to the pheromone alone had been observed.

Peripheral interactions at the receptor level within the pheromone subsystem and interactions during central processing between pheromone and general odorant subsystems result thus in complex integration of both informations.

Plenary Symposium 3 OP17

A challenging task for a male noctuid moth: scenting the conspecific female sex pheromone in the background of plant volatiles

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Mating is the most critical factor in an insect life to transfer its own genetic material. But finding conspecifics in a complex habitat, which consists of a large variety of different cues, is a difficult task. Like in many other insects, females of the noctuid moth Heliothis virescens (Hv) therefore release chemical cues, a species-specific pheromone blend, to attract mating partners. During evolution males have evolved extremely specialized antennae to detect these volatiles. In Hv one of the pheromone components is the major sex pheromone component Z11-16:Ald. Since there is an enormous amount of volatiles being released from the living environment, we addressed the question, if plant volatiles affect the detection of Z11-16:Ald in male Hv . We could show, that certain plant-related odorants decrease the detection at the level of the olfactory sensory neurons (OSNs) expressing the Z11-16:Ald-receptive odorant receptor HR13. Moreover, via in vivo and in vitro calcium imaging we investigated this suppression effect in a region of the magroglomerular complex of the antennal lobe, where Z11-16: Ald-receptive OSNs terminate. Furthermore, we are in the process of examining the behavioral relevance of this interaction in wind tunnel experiments.

Sex pheromone and food search in caterpillars

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Sex pheromones are released by adults of a species to elicit a sexual interaction with the other sex of the same species. In insects, the first step in pheromone recognition is interaction of the components with pheromone-binding proteins (PBPs) to make these hydrophobic molecules able to cross the aqueous lymph to reach the membrane bound receptors. Although these PBPs were thought to be adult specific, we unexpectedly identified 3 PBPs expressed in caterpillar antennae of the crop pest model *Spodoptera littoralis*, while exploring its chemosensory transcriptome. In addition, we showed by electrophysiology that the olfactory sensilla carried by the caterpillar antennae are sensitive to the pheromone. We then conducted behavioural experiments to explore the possible function of the sex pheromone in caterpillar. First, we demonstrated that *S. littoralis* larvae are attracted by the female sex pheromone. Then, we demonstrate that the larvae are preferentially attracted to a food source when it contains the sex pheromone main component. A possible interpretation of these results is that the sex pheromone is used to promote food search in caterpillars, opening potential new routes for insect pest management.

Larval and adult pheromones modulate larval food choice in Drosophila

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Insects use chemosensory cues to feed and mate. In Drosophila, the effect of pheromones has been extensively investigated in adults, but rarely in larvae. Our data show that both larval and adult pheromones can specifically influence larval food search and the choice of a pupariation site. This indicates that these substances affect the dispersion and survival of Drosophila species in nature.

We recently showed that the colonization of natural food sources by *Drosophila buzzatii* and *D. simulans* species partly depends on species-specific chemical cues left in the food both by larvae and adults. In these two species, we identified some of the pheromones and measured their influence on larval food preference and pupariation behavior. We are now focusing our effort on *D.melanogaster* larval behavior and testing the behavioral effect of several larval-produced compounds. We have also started to map the neural pathway underlying their perception. We also compare the behavioral responses of transgenics (invalidated for cells involved in pheromonal perception) and several wild-type lines. We will present our most recent data obtained with these different species and populations.

Starvation modulates coding of sex and habitat chemosensory signals in Drosophila

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Modulating behaviour according to physiological requirements is crucial for animal survival and reproduction. In the fruit fly *Drosophila melanogaster*, the male-produced pheromone *cis*-vaccenyl acetate (cVA) regulates many aspects of social and sexual behaviour. Here we show that starvation differently affects cVA perception in male and female flies. While males become attracted to cVA when starved, females are only attracted when fed. This sexual dimorphic effect of starvation on behaviour is correlated with the activity in the fly antennal lobes (ALs), the first olfactory centre in the insect brain. Starvation increases sensitivity to food odours (vinegar) in both males and females. In contrast, using functional imaging we could show that starvation reduces the response to cVA in the ALs of females. The effect of feeding on female attraction to cVA is mediated by the insulin-signalling pathway within olfactory neurons converging onto specific glomeruli in the ALs, including glomeruli innervated by cVA receptive OSNs, DA1 and DL3, as well as the glomeruli DM1, VA2 and VM2 that respond to vinegar. Female sexual receptivity is also dramatically affected by starvation. However, this effect is independent from the insulin-signalling pathway. This suggests that starvation regulates pheromone perception and sexual receptivity through different mechanisms. In conclusion, insulin signalling controls pheromone perception in *Drosophila* females to match their sexual receptivity in response to changes in food availability.

Abstracts of Parallel Symposium 3

Friday, September 12th

14:30-15:30

Parallel Symposium 3

Modulation of olfactory sensitivity and related behaviors by metabolic status

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The causal relationship between olfactory sensing and food intake is obvious: olfaction plays an important role in food selection/intake control, and the internal physiological status, especially the nutritional one, has profound effects on odor perception. Following the evidence that many receptors to hormones/peptides related to feeding are expressed in peripheral olfactory tissues (olfactory mucosa and bulb) and contribute to detect changes in both peripheral/central hormone and nutrients levels, it has becoming urgent to understand how metabolic state relates to odor perception at the most peripheral level due to its possible role in dietary and eating behaviors control.

In this symposium, we will discuss if and how metabolic disorders, that disrupt energy balance and potentially food intake control, are able to modulate odor signal treatment leading to alteration of olfactory-driven behaviors. Using animal models responding to various diets or hormonal treatments mimicking metabolic disorders encountered in humans, we will show that these dietary imbalances induce long lasting structural and functional changes at the very first levels of odorant coding that deeply and sometimes irreversibly impact olfactory performances. We will explore some of the possible molecular mechanisms that are responsible of this close relation through various *in vivo* and *in vitro* convergent methodological approaches.

Such fundamental connection between olfactory sensitivity and metabolic state is of a great interest, particularly in obesity context, and opens a lot of possibilities to use it either as early indicator of metabolic disorders or as original pathway to develop therapeutic strategies.

Hyperlipidemic Diet Causes Loss of Olfactory Sensory Neurons, Reduces Olfactory Discrimination, and Disrupts Odor-Reversal Learning

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Energy homeostasis is achieved through a coordinated regulation between the peripheral organs and the brain. We currently hypothesize that the olfactory system is intimately linked with the endocrine system; not only does it serve as a detector of external chemicals to yield an internal depiction of our external environment, but it may serve a secondary function as an internal sensor of the chemistry of metabolism. We will present electrophysiological evidence for the detection of several important metabolic molecules that alter the biophysical properties of mitral cells of the olfactory bulb, including glucose, insulin, and glucagon-like peptide (glp-1). In response to diet-induced obesity and hyperglycemia we observe changes in mitral cell activity, loss of olfactory sensory neurons and glomerular targets, and reduced odor discrimination by olfactometry. Proinflammatory responses were activated in the main olfactory epithelium whereby high-fat feeding drove an increase in microphages and an increase in neuronal death by apoptosis, which led to reduced connections from the epithelium to the olfactory bulb. We have found that switching animals to control diet after they were originally reared to high-fat diets allowed a return to normal body weight and serum chemistry but olfactory behaviors and circuitry were not restored. At the heart of this detection may be a voltage-dependent potassium channel, Kv1.3, that is predominantly expressed in the mitral cells of the olfactory bulb and for which targeted deletion infers a resistance to both diet- and genetic-linked obesity. We have begun therapeutic strategies for diabetes and obesity using intranasal, osmotic mini-pump, and nanoparticle technologies to block the vestibule of Kv1.3 to modify metabolism.

This work was supported by the National Institutes of Health (NIH) grants R01DC003387, R01DC013080 from the NIDCD, and ARRA Summer Experience for High School Educators, and the Council for Creativity and Research (CRC) from FSU.

Diet-induced obesity impairs olfactory-driven behaviors concomitantly with alterations of cellular dynamics and homeostasis in olfactory tissues

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Food-induced obesity is associated with chronic food intake disorders and binge eating episodes. Food intake depends on an interaction between homeostatic regulation of the energy balance and hedonic signals, of which olfaction is a major sensory determinant. The influence of this induced obesity on olfactory function remains a matter of debate. The objective of this study was therefore to investigate the impact of obesity induced by a long-term high fat-high sugar diet on olfactory performance and on some factors implicated in cellular dynamics and homeostasis of the olfactory mucosa (OM) and bulb (OB), the initial levels of message coding, in obesity-prone adult Sprague- Dawley rats (OP). Compared to their lean counterparts fed with a control diet (OR), OP rats displayed a decreased odor threshold but poor performance in olfactory-driven sniffing and hidden cookie retrieval behaviors, associated with learning and memory deficits. Furthermore, the influence of insulin on food-seeking behavior observed in OR rats was abolished in OP ones. Concomitant with these behaviors modifications, our data revealed a modulation of metabolic related factors in the OM of OP rats: decreased levels of insulin receptor, monocarboxylate (MCT1) and glucose transporters (GLUT 3 & 4), but no modulations of leptin and ghrelin receptors. Furthermore, OM cellular dynamic in OP rats seemed strongly impaired through higher levels of apoptotic factors and lower levels.

This work was supported by the GLN (Groupe Lipids et Nutrition).

Modulation of olfactory responsiveness by endocrine signals

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The sense of smell significantly affects the amount of consumed food as well as the frequency of food intake. Most animals, such as rodents, rely on the olfactory system for their daily foraging. The necessity to find suitable food sources is particularly urgent when the internal energy reservoir, the body fat content, is running low. The level of body fat is reflected by the concentration of two adipokine hormones, adiponectin and leptin, that are considered as long-term modulators of energy balance. Whereas the leptin concentration positively correlates with the body fat content, the level of adiponectin in the circulation is increasing when the fat reserves are declining. The finding that olfactory sensory neurons (OSNs) have a receptor for adiponectin has led to the hypothesis that adiponectin may alter their responsiveness. In fact, we have found that nasally applied adiponectin renders OSNs more responsive to odorants. This increased activity was also reflected on the next level of olfactory information processing, in the olfactory bulb; after hormone pre-treatment significantly stronger neuronal responses to odor stimuli were registered at receptor-specific glomeruli, indicating that the stronger response in the periphery was in fact conveyed to the brain. An application of leptin, in contrast, did not lead to any change in the responsiveness of olfactory neurons to odorants.

Food finding and food consumption is also crucial in an acute hunger situation. The gastric hormone ghrelin is known as an indicator for acute hunger and supposed to elicit food intake behavior. In our approaches to assess whether ghrelin may also affect OSNs we found that the receptor for ghrelin, GHSR1a, is expressed in the olfactory epithelium. Moreover, monitoring the olfactory responsiveness revealed that after a pre-treatment with nasally applied ghrelin, a standardized olfactory stimulus activated a significantly higher number of OSNs, and also led to an increased activity of the corresponding glomeruli. The short-term satiety hormone PYY, in contrast, did not show any effect on the olfactory responsiveness. Together the results demonstrate that hormones which represent conditions of scarce energy reserves or conditions of acute hunger both render the olfactory system more responsive to odor stimuli and may thereby contribute to cover the energy demand by an improved discovery of food sources.

This work was supported by the Deutsche Forschungsgemeinschaft.

Imaging odor-evoked activity in the olfactory bulb of ob/ob mice using Manganese-Enhanced MRI

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The olfactory system is crucial for feeding behavior: it allows the processing of olfactory cues related to food location and palatability. Interestingly, receptors to anorexigen (leptin and insulin) and orexigen (ghrelin) hormones found in the hypothalamus are also expressed in the main olfactory bulb (MOB), the first stage of olfactory processing in the brain, suggesting that feeding state has an impact on odor representation. In order to study the relationship between olfaction, feeding and obesity, we tested the effects of obesity on the MOB activity in vivo.

Ob/ob mice are deficient in leptin from birth and are widely used as a murine model for obesity since they are hyperphagic and rapidly obese. We used a functional neuroimaging technique, Manganese Enhanced MRI, to monitor food odor-evoked activity in the MOB of these mice. MEMRI uses manganese as a contrast agent. This ion is an analog of calcium and penetrates into the olfactory neurons that are activated by odorants. Then manganese is transported and accumulates in specific regions of the MOB making possible MEMRI recordings of spatial activity in the MOB. We found that the number of activated pixels in the MOB in control conditions with no odor stimulation is higher in ob/ob mice compared to wild type mice (each group n=5). We also observed that the number of food odor-activated pixels is higher in the ob/ob mice (each group n=5). Moreover, injection of leptin strongly reduces the number of food odor-evoked pixels (each group, n=4) in ob/ob mice. Thus leptin strongly impacts odor-induced activity in the MOB.

One mechanism that could explain this hypersignal in ob/ob is the presence of a local inflammation. It was shown that reactive microglia transports manganese at high rates. We quantified mRNA expression of neuronal (OMP), astrocytic (GFAP) and microglial (IBA1) molecular targets by RT-PCR, but did not find any significant changes between ob/ob (n=10) and control mice (n=10) indicating that neuronal activity changes detected by MEMRI are odor-specific and not due to local inflammation due to obesity.

Negative impact of high fructose diet on olfactory abilities

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Fructose is excessively present in modern Western industrial diet, especially via high fructose corn syrups (HFCS), found in sweetened solid food and beverages. The increase in fructose consumption over the last 40 years coincides with the rise in prevalence of type 2 diabetes mellitus (2DM) reaching 6.8 % in Europe and 9.6 % in North America in 2013. It appears that patients with 2DM show abnormal olfactory abilities, even though it is not clear yet whether olfactory dysfunctions are due to 2DM in itself or secondary pathologies such as micro- or macro-angiopathy.

Over the past few years, several studies showed that nutritional status influences olfactory abilities. Indeed fasting increases whereas satiation decreases olfactory capacities both in animals and humans. Moreover hormones implicated in the regulation of energy homeostasis can act on the olfactory system, both at central and peripheral levels. But few studies have been conducted on the influence of metabolic disorders induced by diet on the olfactory system.

Here we investigated the effects of a fructose enriched diet (known to induce rapidly 2DM) on the olfactory system in mice. Young adult mice (age 5 weeks) were given a 60%-fructose diet (HFruD for high-fructose diet) for 4 or 8 weeks. We then measured the metabolic status of the animals (glycemia, weight gain and food intake) and tested their olfactory abilities. HFruD animals displayed a hyperglycemia after 4 weeks of fructose diet (160 mg/dl +/- 4.4 *vs* 116 mg/dl +/- 16.7). Two behavioral approaches were used to test animals' olfactory abilities: the habituation / dishabituation test and the buried food test (to assess olfactory abilities for neutral odors and food odor respectively). After 4 weeks of fructose diet, HFruD animals showed a decrease in the dishabituation ratio (1.4 +/- 0.2 *vs* 3.8 +/- 0.5) indicating that HFruD animals could not discriminate between two odorants anymore. Likewise the mean food finding time in HFruD animals was longer compared to control animals (34.4 s +/- 4.7 vs 17.4 s +/- 2.3). Those effects persisted after 8 weeks of HFruD. Finally we quantified the effect of HFruD on the olfactory mucosa by using electro-olfactogram (EOG). The amplitudes of odorant responses were partly reduced and their kinetics altered for HFruD animals compared to control animals.

In conclusion, consumption of a 2DM-inducing-diet enriched in fructose leads to a decrease in olfactory capacities, both at behavioral and functional levels.

Abstracts of Parallel Symposium 4

Friday, September 12th

15:30-16:30

Parallel Symposium 4

Taste receptors and diet in wild and domestic animals

Eugeni Roura[1], Scott McGrane[2]

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Most of the research regarding the taste system has been and it still is today carried in humans or laboratory rodents. However, our understanding of the specificities of taste in different animal species other than humans and rodents has gained significant depth in the recent years. The taste receptor repertoire related to a number of wild, companion and farm animals such as the genre of Carnivora, domestic cat, dog, pig, chicken and domestic herbivores among others, has been updated over the last years. In addition, an evolutionary perspective seems to link the vertebrate taste system with dietary needs. The Symposium will update our current understanding of the taste system in wild, companion and farm animals and will emphasize the links related to dietary adaptations.

Taste adaptation to diet in Carnivora

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Each animal species lives in a separate sensory world. A dramatic example of this occurs for the sense of taste where the interrelationships between sensory perception and diet choice are clearly evident. The major hypothesis explaining the existence of a small number of basic or primary taste qualities is that these qualities evolved to detect and motivate consumption of critical nutrients and to prevent ingestion of potential poisons. Our studies of the order Carnivora have the goal of understanding how taste receptors and taste perception in different species are related to different feeding ecologies. For example, some Carnivora species are obligate carnivores (e.g. cats) whereas others are strictly herbivorous, sometimes feeding on virtually a single plant (e.g. giant panda). Many years ago, we demonstrated that domestic and wild cats (*Felis* and *Panthera*) are indifferent to all sweeteners tested but are highly responsive to certain amino acids. Following the discovery of the major sweet taste receptor, the T1R2 + T1R3 dimer, we demonstrated that the cat's indifference to sweeteners can be explained by the pseudogenization of the *Tas1r2* gene which encodes the T1R2 receptor. From this, we reasoned that other exclusively meat-eating species might also have an inactive form of this gene. Sequencing of the entire coding region of *Tas1r2* from twelve carnivore species revealed that seven of these species, all exclusive meat eaters, had independently fixed a defective *Tas1r2* allele. Behavioral tests of two of the genotyped species, the Asian otter (defective *Tas1r2*) and the spectacled bear (intact *Tas1r2*), were consistent with the genetic findings. These results indicate that the independent loss of a functional *Tas1r2* is widespread among obligate carnivores probably due to the relaxation of selective pressures maintaining receptor integrity. In more recent behavioral and molecular studies with giant pandas, animals that do not consume plants with abundant simple sugars, we found sweet taste perception is fully functional. Although giant pandas thus retain an avidity for sweet compounds, genetic evidence suggests it has lost umami taste perception. Finally, looking more broadly, it appears that many mammalian species that have returned to the sea (e.g. sea lions, dolphins, whales) may have lost function for several, perhaps all, taste quality perception. These data dramatically illustrate how plastic the taste system is and how it has adapted to changes in diet as species have evolved.

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Physiological relevance of the Taste 1 Receptor (T1R) family in the domestic cat and dog

Scott McGrane[1], Andy Taylor[1]

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The T1R family of Class C G protein-coupled receptors was first discovered in the peripheral gustatory system (taste) and later also in other tissues and organs (such as in the nasal epithelium, gut, pancreas, liver, kidney, testes and brain) in various mammalian species. There are three proteins in the family, T1R1, T1R2, and T1R3, encoded by their respective genes, *Tas1r1*, *Tas1r2*, and *Tas1r3*. T1R2 combines with T1R3 to form a heterodimer (T1R2-T1R3) that binds sugars and other sweeteners, while T1R1 also combines with T1R3 to form a heterodimer (T1R1-T1R3) that binds L-amino acids (Treesukosol et al., 2011).

Cats and dogs belong to different branches within the order Carnivora, with most species in this order not actually being totally carnivorous, despite their name. The domestic cat (*Felis catus*) is an obligate carnivore and naturally eats a very low carbohydrate diet, whereas the domestic dog (*Canis familiaris*) is a semi-carnivore and has a varied diet. It has been proposed that the strictly carnivorous cat has evolved to recognise and metabolise compounds from meat sources, while the dog has developed to recognise and metabolise compounds from both meat and non-meat sources, such as fruits.

Cats exhibit no preference for sugars or artificial sweeteners; however, they do show nerve responses to certain amino acids and nucleotides as well as behavioural preferences for these compounds (Bradshaw, 1991; Bradshaw, 2006). The cat *Tas1r2* is an unexpressed pseudogene; hence a functional sweet taste receptor heterodimer cannot form, which is necessary for detection of sweet stimuli. In cats the T1R3 receptor is functional (Li et al., 2005) and more recently it was reported that T1R1 is also functional, with the two forming a heterodimer to produce the umami receptor heterodimer (McGrane et al., 2013). In contrast, dogs exhibit preference for sugars, artificial sweeteners and also certain amino acids (Bradshaw, 1991; Bradshaw, 2006), consistent with them being semi-carnivores. Dogs have functional T1R1, T1R2, and T1R3 taste receptors.

In addition to the taste system, it has also been shown more recently that both cats and dogs express T1R3 in a subset of intestinal epithelial cells, and dogs not cats, express T1R2 in these same cells (Batchelor et al., 2011).

The current status of this fascinating field of research, which opens a window into the dietary preferences and sensory world of domestic cats and dogs, will be reviewed in this presentation.

Taste receptor expression and function in omnivorous farm animals

Eugeni Roura[1]

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Nutritional chemosensing is a scientific discipline involved in understanding the role of nutrient sensors, including taste receptors (TR), in nutrient homeostasis. Consequently the manipulation of taste may have profound implications on the production of farm animals. Emerging anatomic, genomic and behavioural observations in birds show potential for high taste acuity (Roura et al., 2013). For example, the analysis of chicken (*Gallus gallus domesticus*) and turkey (*Meleagris Gallopavo*) genomes shows high homology of several taste and nutrient sensor genes to the mammalian orthologues. However, no sign of the mammalian sweet-related receptor T1R2 was found in these two species. In addition, the bitter taste receptor gene (Tas2R) repertoire is predicted to have only 3 and 2 members in chickens and turkeys, respectively. Potential implications relevant to poultry nutrition such as taste-driven calcium or amino acid specific appetites warrant further considerations.

In pigs (*Sus scrofa*) we performed a systematic TR gene analysis which identified twenty-six nutrient sensing genes expressed in the oral cavity (de Jager et al., 2013). The study uncovered the relative abundance of twelve receptors with known affinity to dietary fatty acids (GPR40, GPR41, GPR43, GPR120 and GPR84), simple carbohydrates (the dimer T1R2/T1R3) and amino acids/peptones (T1R1/T1R3, mGluR1, mGluR4, GPC6A and CaSR). In general, porcine nutrient sensors are highly conserved compared to humans and laboratory rodents and even chickens. However, the comparison of the bitter taste system showed larger variability. The biodiversity of the bitter taste system seems to have co-evolved with the need to adapt to these environmental threats such as potential toxic plant compounds often unique to specific ecosystems. One of our most recent studies (da Silva et al., under review) has characterized the TR and nutrient sensor gene polymorphisms (SNP) across 74 genomes belonging to 14 different pig breeds. The population genomic study shows high variability of the Tas2R family linked to breeds of specific geographical origins. Thus, our data suggests that the porcine bitter taste is a plastic trait, possibly associated with the ability of pigs to adapt to diverse environments.

In conclusion, pigs and chickens have a fully developed taste and nutrient sensing system. While the nutrient sensors are highly conserved, in contrast, the Tas2R repertoire shows high diversity both between species and between pig breeds. The porcine Tas2R polymorphisms may constitute additional evidence of their role in environmental/dietary adaptations.

Relating taste receptor function to feeding ecology and evolution: a cautionary tale

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With the increased availability of genomic data, it is now possible to ask evolutionary questions about interspecific differences in taste function—e.g., how diet influences the evolution of taste receptors. This comparative approach to taste offers great promise, but one must keep in mind that (a) there are probably many as-of-yet unidentified taste receptors, and (b) not all interspecific differences in taste receptor expression reflect evolutionary adaptations. I will illustrate this point by discussing recent work on the sugar (T1r2/T1r3) and "bitter" (T2r) taste receptors.

First, T1r3 KO mice are indifferent to sugars during their initial exposure, but nevertheless condition strong and enduring preferences for sugars following dietary exposure. Further, my colleagues and I recently discovered that T1r3 KO and T1r2/T1r3 KO mice display normal cephalic-phase insulin release in response to oral stimulation with sugars. Thus, one must be careful assuming that the presence of a functional T1r2/T1r3 taste receptor is necessary for a species to generate adaptive behavioral and physiological responses to sugars. Indeed, it is likely that there are additional taste receptors for simple and complex carbohydrates.

Second, vertebrate species vary in the number of T2rs that they express. Because plant tissues contain a relatively high abundance of "bitter" and potentially toxic compounds, it has been hypothesized that natural selection would have favored the evolution of a more diverse repertoire of T2rs in herbivorous animals. Implicit in this hypothesis is the assumption that animals with a relatively large number of T2rs have an enhanced ability to (a) detect poisonous compounds and (b) discriminate between harmful and harmless bitter-tasting foods. I will present several lines of evidence that contradict this assumption, and suggest that at least some interspecific differences in T2r expression reflect phylogenetic constraints rather than adaptive specializations.

Tuning breadths and receptive ranges of avian bitter taste receptors

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The ability of vertebrates to taste the numerous and frequently toxic bitter compounds present in nature is important for the quality assessment of food and hence, the survival of species. Recognition of the structurally diverse bitter compounds is facilitated by specialized G protein-coupled receptors, the Tas2rs. Interestingly, the numbers of putatively functional *Tas2r*genes deviate considerably among vertebrate species ranging from very few, e.g. in teleostean fish and some birds such as chicken and turkey, to ~50 *Tas2rs* in frog. Based on the comparatively low number of oral taste buds, low salivation, and a lack of mastication, it was hypothesized that birds may possess an inferior taste system. Indeed, the absence of a functional *Tas1r2* gene, coding for the specific sweet taste receptor subunit and the small repertoire of only three *Tas2rs* may indicate reduced importance of the tasting abilities in chicken. However, other bird species such as the zebra finch with ~7 or the white-throated sparrow with ~18 *Tas2rs* possess more putatively functional *Tas2r* genes in their genomes approaching gene numbers found in mammalian species.

In order to test whether a low *Tas2r* gene number in birds may correlate with a lower importance of bitter taste and to investigate the receptive ranges of avian Tas2rs we cloned and functionally expressed selected Tas2rs of the three bird species chicken, turkey and zebra finch. After transient transfection of the Tas2r-constructs into HEK 293T-Gα16gust44 cells, calcium imaging analyses using a panel of 46 different bitter compounds were performed.

Our screening of the 3 functional chicken Tas2rs representing the entire bitter taste receptor repertoire of this species revealed that all three Tas2rs represent broadly tuned receptors recognizing large spectra of bitter substances. The two turkey Tas2rs, again representing the entire Tas2r repertoire of this species, are both similarly broadly tuned. Moreover, not only the breadth of tuning is conserved among chicken and turkey receptors, the two pairs of orthologous receptors exhibited an almost identical spectrum of agonists even though the species separated ~40 million years ago. Ongoing experiments with four of the seven zebra finch Tas2rs indicate that at least some of the receptors show a narrow tuning breadth.

In summary, we conclude that a low number of *Tas2r* genes does not predict a reduced importance of the bitter tasting abilities in the corresponding species, whereas a higher number of functional *Tas2rs* may allow the development of more specialized narrowly tuned receptors.

Experience Affects Mouse Sweet-Taste Phenotypic Behavior

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A major locus influencing consumption of solutions of sodium saccharin (Sac) solutions discovered in crosses between C57BL/6J (B6) and DBA/2J (D2) mouse strains had been earlier suggested to exert its effect via its impact on motivational systems. More recently, peripheral sweet receptor differences are invoked to account for variation in sweet taste behavior among inbred mouse strains. The present study emphasizes the role of experience in sweet preference behavior. Naïve D2 mice, a sweet sub-sensitive strain, show little or no preference for 0.01 to 0.10 M sucrose, which sweet-sensitive B6 mice prefer. Exposure of D2 to 0.3 M sucrose for 48 h in a 2-bottle preference test (Suc group) resulted in a modest 70% preference for 0.03 M and a strong 95% preference for 0.10 M sucrose. Preference for 0.10 M sucrose was higher in Suc mice than in control (Ctrl, non-exposed) and in a group previously exposed to 20 mM Sac. Preference for 10 mM Sac by Suc group mice was also higher (95%) than the 75% preference in Ctrl and Sac group mice. These results emphasize the importance of environment in the development of the mouse sweet preference phenotype. A sub-sensitive strain can be made sweet-sensitive by exposure to a high concentration of sucrose for a 48 h period. The results will be compared to those previously obtained with B6 mice, which, following excessive sucrose consumption, did not change sweet preference behavior. Gustatory cortical (GC) plasticity with regard to hedonic sign has been demonstrated by Accolla and Carleton (2008) in their study of conditioned aversions in rats. We propose that the reinforcing effects of consumption of sugars may similarly increase GC representation of gustatory sensory afferents, augmenting afferent signaling value especially in sweet sub-sensitive strains. Other studies with a different sweet sub-sensitive strain, 129/J, also show that exposure to high caloric sweeteners in liquid form may alter subsequent intake of sweet solutions in mice. If human sugar intake similarly increases with exposure to caloric sweeteners, it would have important relevance to public health issues such as controlling obesity and diabetes.

Abstracts of Parallel Symposium 5

Saturday, September 13th

09:00-10:00

Parallel Symposium 5

Decoding and mapping underwater olfaction: recent progress in zebrafish and *Xenopus*.

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The olfactory systems of zebrafish and *Xenopus* are amenable to genetic, anatomical and physiological experiments and have thus become favored models to study the neurobiology of olfaction. Studies using these species have advanced our understanding of how the olfactory system detects environmental odor molecules, how the information contained in these molecules is encoded and mapped in the olfactory bulb, and ultimately how it is processed in higher brain regions. These studies span all levels of analysis – from genes to behavior. Several organizational and functional features of the zebrafish and *Xenopus* olfactory systems appear to be shared, but few attempts have been made at comparing the olfactory systems of these model species.

This symposium brings together several experts of the field of aquatic olfaction that will discuss recent experimental progress made in these two species. The most important aspect will be to highlight similarities but also differences in olfactory coding mechanisms in the two animal models. This symposium will be an excellent opportunity to establish a comparative framework that fully describes olfaction in aquatic vertebrates.

Olfactory neural circuitry mediating reproductive behavior in zebrafish

Yoshihiro Yoshihara[1]

[1] *RIKEN Brain Science Institute, Japan*

Zebrafish has become one of the most useful model organisms in neurobiology. In addition to its general advantageous properties (external fertilization, rapid development, transparency of embryos, etc.), zebrafish is amenable to various genetic engineering technologies such as transgenesis, mutagenesis, gene knockdown/knockout, and transposon-mediated gene transfer. Our transgenic approach unraveled two segregated neural pathways originating from ciliated and microvillous olfactory sensory neurons (OSNs) in the olfactory epithelium to distinct regions of the olfactory bulb, which likely convey different types of olfactory information. Furthermore, the two basic principles (one neuron - one receptor rule and axon convergence to target glomeruli) are essentially preserved also in zebrafish, rendering this organism a suitable model vertebrate for the olfactory research (Sato et al, 2005; Koide et al, 2009; Miyasaka et al, 2014). In this talk, I will summarize recent advances in our knowledge on functional architecture of the zebrafish olfactory circuits mediating specific odor-induced behaviors. In particular, I will focus on our recent findings on the olfactory neural circuitry mediating sexual behavior in zebrafish. In teleost fishes, prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) has been proposed as a female sex pheromone that evokes reproductive behavior in males. However, it remains largely unknown which olfactory receptor and which type of OSNs are activated by $PGF_{2\alpha}$ and how its pheromonal information is transferred from the olfactory epithelium to the olfactory bulb and further into higher brain centers to evoke the sexual behavior. To identify $PGF_{2\alpha}$-activated OSNs and central neurons along the olfactory pathway, we used immunohistochemical labeling of phosphorylated Erk as a neuronal activation marker and GCaMP calcium imaging in transgenic zebrafish. Upon $PGF_{2\alpha}$ stimulation, neuronal activation was detected in a small population of ciliated OSNs in the olfactory epithelium, two ventromedial glomeruli in the olfactory bulb, and several nuclei in the ventral forebrain and hypothalamus. In addition, double in situ hybridization using c-Fos and olfactory receptor probes revealed two candidate receptors for pheromonal $PGF_{2\alpha}$. These results provide molecular and anatomical bases for the olfactory neural circuitry mediating sex pheromone-induced reproductive behavior.

Birth and Migration of Sensory Neurons in the Adult Zebrafish Olfactory System

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Individual olfactory sensory neurons (OSNs) express only a single olfactory receptor (OR) gene from a large and diverse genomic repertoire. Typically, OSNs expressing the same OR are confined to distinct expression domains in the olfactory epithelium (OE) and converge onto the same glomeruli in the olfactory bulb; features that are conserved in organisms as disparate as vertebrates and invertebrates. Because of their direct exposure to the environment, OSNs have a limited lifetime and need to be continuously renewed even in adult organisms. In the rodent OE newborn OSNs are supposed to be generated from stem cell populations located in the basal OE and to migrate towards more apical positions as they adopt functional maturity.
In the zebrafish OE, OR expression patterns can be recognized as concentric domains with OR-specific diameters and distributions. Contrary to the rodent OE, zebrafish OSNs are not predominantly generated in the basal OE but from two distinct zones of proliferative activity located at the inner curves between olfactory lamellae and at the peripheral sensory/non-sensory boundary of the olfactory rosette. Incubation with the proliferation marker BrdU and expression analysis of proneuronal and neuronal markers allowed us to track maturation of newborn OSNs after they exit mitosis. OSNs begin to express molecular markers of OSN differentiation, including OR genes, in close proximity to the sites of active neurogenesis. As they mature, OSNs invade the sensory epithelium by active migration and form an OR-specific annular pattern, suggesting that the observed similarity of zonal OR expression patterns in rodents and zebrafish are analogous features and generated by different mechanism.

Expression of ancestral V2Rs shifts from the main olfactory epithelium of tadpoles to the water nose of adult Xenopus laevis

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In mammals olfactory receptor families are segregated into different olfactory organs, chief among them the main olfactory epithelium (MOE) and the vomeronasal organ (VNO). In contrast, teleost fish olfactory receptor families are intermingled in a single sensory surface. To what extent such differences influence the coding and discrimination abilities of the respective olfactory systems is unclear, and the evolutionary path toward such segregation is unknown. Amphibians are early diverging tetrapods compared with mammals, and occupy an intermediate evolutionary stage concerning the water-to-land transition. Consequently, their analysis may shed light on this transition from shared sensory surface to segregated subsystems. We report here that a major olfactory receptor family of *Xenopus laevis* , V2Rs, is expressed in both olfactory organs of tadpoles, with the VNO expressing more 'modern', later diverging V2Rs, whereas more 'ancient', earlier diverging V2Rs are expressed in the MOE, together with the V1R family. Furthermore, amphibians such as *Xenopus* make their own ontogenetic transition from an obligate (tadpole) to a facultative aquatic stage in adults. During metamorphosis the MOE of *Xenopus* tadpoles transforms into an air-filled cavity (principal cavity, air nose), whereas a newly formed cavity (middle cavity) takes over the function of a water nose. We report here that larval expression of 'ancient' V2Rs is gradually lost from the main olfactory epithelium as it transforms into the air nose. Concomitantly, 'ancient' V2R expression begins to appear in the newly forming water nose. Responses to amino acid odorants are present in the tadpole MOE, and show the same transition, disappearing in the transforming air nose and concomitantly appearing in the new water nose, consistent with the hypothesis that amino acid responses may be carried by V2R receptors. Interestingly, 'ancient' v2r genes are expressed in a basal expression zone both in tadpoles and adults, so this feature of V2R expression is stable during the migration of expression from one olfactory epithelium to another during metamorphosis.

Sulfated steroids are chemosensory stimuli in both the main and accessory olfactory system of an amphibian

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Sulfated steroids are negatively charged molecules with low molecular weight and low volatility that have been shown to be present in mouse urine. They trigger responses in a large number of sensory neurons in the mouse vomeronasal organ, where they may act as pheromones. Using functional calcium imaging in acute slices of the olfactory organ and the olfactory bulb, we found that some pregnanolone-derived (P-mix) and oestrogen-derived (E-mix) sulfated steroids elicit large Ca^{2+} responses in sensory neurons of *Xenopus laevis*. Surprisingly, these compounds activate sensory neurons in both the main olfactory epithelium (MOE) and the vomeronasal organ (VNO). However, pregnanolone- and oestrogen-derived steroids showed a differential specificity in the two olfactory organs. Pregnanolone-derived steroids activated sensory neurons in both the MOE and VNO, oestrogen-derived steroids only activated neurons of the MOE. Individual sensory neurons in both organs generally responded to only one of the two steroid mixtures (P-mix or E-mix). Nonetheless, most individual sensory neurons responded to more than one steroid within a mixture, indicating that individual neurons are tuned to detect the class of sulfated steroids (either P-mix or E-mix), rather than single steroids within the same class. Our data support the view that steroid metabolites could be ancestral vertebrate chemosensory stimuli, and provide the basis for further investigations of their physiological role in amphibians.

[Supported by DFG Schwerpunktprogramm 1392 (I.M) and Cluster of Excellence and DFG Research Center Nanoscale Microscopy and Molecular Physiology of the Brain (I.M).]

Taste aversion in Zebrafish juveniles

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The sense of taste helps acquiring and maintaining an appropriate feeding behavior, allowing to select good food and reject deleterious substances. For instance, sweet taste permits the identification of energy rich nutrients, and bitter warns against the intake of potentially poisonous chemicals. Taste impairment (dysgeusia) occurs in several diseases and it can give rise to weight loss through reduced appetite and altered patterns of food intake. On the other hand, obesity in human may be linked to exacerbated appetite for sweet substances, often acquired during childhood. As a consequence of the important role of taste in nutritional behaviour in healthy humans and patients, considerable research work has recently been focused on the mechanisms of development, function and regeneration of the gustatory system. The reasons why a given animal selects a specific diet over a large choice of food items are still obscure and remain an intriguing question. Food selection is based at least in part on taste preferences and taste aversion. Taste aversion is often associated with noxious substances, as a large variety of toxic substances have a bitter aversive taste. Thus, taste aversion is crucial in order to prevent animals from ingesting poisonous substances. Numerous studies in human have revealed that taste recognition and preferences are established very early during childhood. Accordingly, in the animal kingdom, taste sensing begins as soon as the animal starts feeding. The question then arises as to how taste aversion is established in the very beginning of life.

We have set up a behavioural test that allows us to determine on which principles zebrafish feeding behavior is based and particularly, what can restrict the quantity of food eaten in a given time. Using this assay, we noticed that taste buds are already functional in 5day-old zebrafish when larvae begin to take food since bitter compounds like denatonium or cycloheximide prevent feeding in larva, consistent with the work of others. Bitter substances elicited an increase of mRNA synthesis of the immediate-early response genes *egr* 1 and *c-fos* , as revealed by in situ hybridization with specific probes. Maximum labeling was observed in cells located on the lips and on the gill rakers. Gustatory areas of the brain were also labelled. Interestingly, when bitter tastants were repeatedly associated to a food reward, zebrafish juveniles learned to ingest food in the presence of the bitter compound. After habituation, the acquisition of acceptance for bitterness was accompanied by a loss of *egr* -1 mRNA specific labelling, suggesting that this early-response gene may serve to signal aversive tastants. The existence of reward-coupled changes in taste sensitivity in humans suggests that our results are relevant to situations in humans.

Abstracts of Parallel Symposium 6

Saturday, September 13th

10:30-11:30

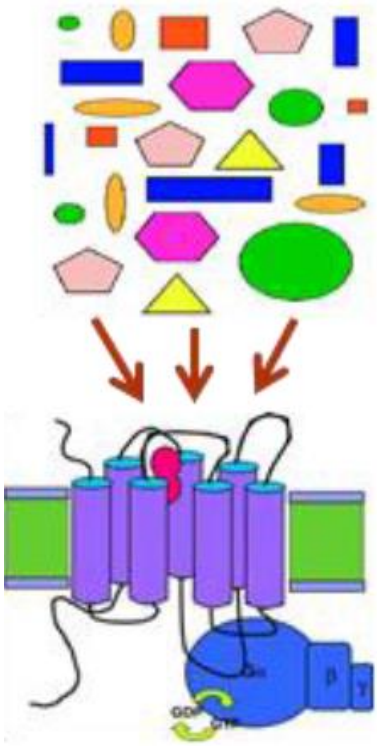
Parallel Symposium 6

Molecular Recognition and Signaling in food sensing GPCRs

Masha Niv[1]

[1] *Hebrew University, Israel*

Diet has an enormous impact on many aspects of our health, and many of the food-related processes are mediated by G protein-coupled receptors (GPCRs).
Recent breakthroughs in understanding of GPCRs signalling and structural biology were celebrated by the 2012 Nobel Prize in Chemistry to Lefkowitz and Kobilka and provide excellent platform for further exploration. In particular, GPCRs mediate the perception of odours, and of bitter, sweet and umami taste perception, and therefore have a crucial role on food choice and consumption. Food is now sometimes viewed as a mixture of hormones, eliciting specific and complicated outcomes in tissues different from the one that produced it. Moreover, it is often the biological transformation of food that generates the GPCR active components. Specifically the talks in the proposed session will discuss new findings on the roles of GPCRs expressed in the gut, as well as identification of new ligands for food-sensing GPCRs.
The suggested symposium tackles the different stages in food-related GPCRs signal transduction, from ligand recognition to intracellular events.



L-amino acid sensing G protein-coupled receptors and their role in food intake sensing

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G protein-coupled receptors (GPCRs) are divided into classes A, B and C receptors based on phylogenetics. The majority of class C GPCRs consists of a large extracellular 'Venus Flytrap' domain and Cystein-rich domain followed by a 7-transmembrane domain. The 'Venus Flytrap' domain is homologous to periplasmic binding proteins which act as amino acid and nutrient transporters in bacteria. Likewise, human class C GPCRs have been shown to binds amino acids, cations and sugars and thus potentially act as nutrient sensors in mammals.

We have paid particular interest to the class C receptor entitled GPRC6A, which will be the focus of the present talk. In 2004 we reported the cloning and tissue expression of the GPRC6A receptor. Using a variety of pharmacological assays at the human, mouse and rat receptor orthologs expressed in *Xenopus laevis* oocytes and mammalian cell lines, we have shown that the receptor is Gq coupled and promiscuously activated by L-amino acids of which the basic L-amino acids L-Arg, L-Lys and L-ornithine are the most potent. In addition, we have shown that the receptor is co-activated by divalent cations such as calcium.

Unfortunately, these endogenous agonists also act on a range of other targets, which decreases their use as pharmacological tool compounds. To study the physiological function we have thus generated a GPRC6A knockout mouse where exon 6 containing the entire 7TM domain was removed. We have shown that the GPRC6A knockout mice had wild-type-like growth rate, body composition and glucose metabolism when fed a regular chow diet. However, when the mice are metabolically stressed by feeding a 60% high fat diet the knockout mice become more obese than their wild-type littermates and show impaired glucose metabolism. Given that the GPRC6A receptor is expressed in the islets of Langerhans and that L-amino acids such as L-Arg are known to increase insulin secretion, we hypothesized that the impaired glucose metabolism could be caused by decreased L-amino acid insulin secretion. However, both *ex vivo* experiments on isolated islets and *in vivo* experiments demonstrate that the GPRC6A knockout mice demonstrate wild-type-like L-Arg mediate insulin release.

Collectively, these studies of our GPRC6A knockout mice indicate a role as nutrient sensor. However, further studies are needed to delineate the precise mechanisms of the observed phenotype.

Developing ligands for GPR41 and GPR43; receptors for short chain fatty acids produced by bacterial fermentation

Graeme Milligan[1]

[1] *University of Glasgow, Scotland*

The microbiota generate large amounts of Short Chain Fatty Acids (SCFA) in the gut by fermentation of non-digestible carbohydrates (Milligan et al., 2014). Although pleiotropic in function, many of the actions of the SCFAs are believed to be mediated by activation of either or both of the pair of G protein-coupled SCFA receptors, FFA2 (also called GPR43) and FFA3 (GPR41) (Milligan et al., 2014). Unravelling the specific contributions of FFA2 and FFA3 has been challenging as both receptors respond with low potency to SCFAs of chain length C1-C5, although with distinct SAR (Hudson et al., 2012a), species orthologues of the receptors show marked differences in both levels of constitutive activity and potency for SCFAs (Hudson et al., 2012a) and receptor knockout mouse models have generated conflicting observations. Despite this there is considerable interest in targetting these receptors in areas relating to metabolic health and inflammatory diseases. However, better validation is required.

To overcome these limitations we have adopted two distinct approaches. In the first we have synthesised a number of FFA2 (Hudson et al., 2013) and FFA3 (Hudson et al., 2014) selective agonists and antagonists. These have been useful in demonstrating roles of these receptors in the release of incretin hormones and in the regulation of lipolysis (Hudson et al., 2013). However, many of these synthetic ligands are allosteric rather than orthosteric in action, and a number of them also only have significant affinity at the human orthologue. We have, therefore, developed a Receptor Activated Solely by Synthetic Ligand (RASSL) form of human FFA2 which no longer responds to SCFAs but instead responds rather to a set of non-endogenously produced ligands. Importantly, the human FFA2 specific antagonist ligands still block this modified receptor and, therefore, we are generating a humanised, knock-in mouse model in which this form of the FFA2 receptor replaces the wild type murine form. Now the non-endogenous ligands activate FFA2 whilst the SCFAs activate only FFA3. This model provides an ideal platform to tease apart the contribution of the individual SCFA receptors.

Molecular recognition of GPCR tastants: bitterness and promiscuity

Masha Niv[1]

[1] *Hebrew University, Israel*

Bitter taste is one of the basic taste modalities, needed to guard the animal against consuming toxic substances which are often bitter. Bitter compounds, recently organized in the BitterDB electronic database, are recognized by bitter taste receptors, a family of broadly tuned G-protein coupled receptors (GPCRs). How do these receptors detect numerous structurally diverse bitter compounds? What are the molecular features of a GPCR that determine its receptive range towards ligands?

The use of different sets of partially overlapping positions within the same binding pocket, and employment of different types of interactions by the same residues, emerge as common strategies that enable bitter taste receptors to bind chemically dissimilar ligands. These distinct interaction fingerprints can be used to identify additional ligands of the receptors. This is of particular interest because of the role of bitter taste receptors in regulating food consumption and in co-evolution with natural toxins. Furthermore, the recently discovered expression of taste receptors in lung, gastrointestinal tract and male reproductive system, highlighted them as novel therapeutic targets. Bitterness of existing drugs opens opportunities for repurposing existing drugs for novel indications by targeting bitter taste receptors.

Intrigued by the extremely broad receptive range of bitter taste receptors, we aimed to unravel the receptor features that determine its promiscuity. To this end, analysis of Family A GPCRs, for which experimental crystal structures were determined, was carried out. Our findings highlight general sequence and structural features, such as hydrophobicity and exposure of the canonical binding site, that correlate with receptor's receptive range and successfully predict the levels of promiscuity of GPCRs that were not included in the derivation of the correlation. These results are discussed in the context of discovery and design of agonists and antagonists for chemosensory receptors.

Regulators of G Protein Signaling (RGS proteins) and tastant signal transduction

David Siderovski[1]

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The molecular basis for sweet, bitter, and umami taste modalities has been established as tastant molecule stimulation of particular T1R or T2R G protein-coupled receptors on lingual taste bud cells. The Siderovski lab at West Virginia University has recently established that the negative regulator RGS21, also expressed in lingual taste bud cells, acts to oppose tastant signaling. We will discuss how this discovery could provide opportunities for manipulating the physiological timing and duration of sweet and bitter gustation.

Intricate or feasible? – Readouts from a genuine type II like human taste cell expressing multiple TAS2Rs

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Recently we have established a stably proliferating human cell line derived from fungiform papillae termed HTC-8. We showed the expression of multiple different human bitter taste receptors (TAS2Rs) in an endogenous fashion, which is in accordance to findings revealed by *in situ* hybridizations of human taste buds. Moreover, we got evidences that HTC-8 feature complex bitter signalling pathways upon stimulation with taste stimuli. The validated expression profile and functional characterization point to a chemosensory cell resembling a type II type human taste cell.

For further research and development we set out to establish read out systems as well as genetic engineering of HTC-8, to cope with the complexity resulting from the endogenous multiple TAS2Rs expression. We will give insights in variable signalling pathways induced by various tastants known as bitter to humans as well as other taste related stimuli.

Artificial sweeteners like Acesulfame K and Saccharin are known to activate TAS2R dependent bitter perception. Hence, we analyzed the response of HTC-8 cells to high intensity sweeteners and were able to show that HTC-8 cells respond via distinct signalling profiles for the two sweeteners, respectively. Moreover, our results reveal that responses to physiologic concentrations were mediated via corresponding TAS2Rs. Thus, HTC-8 cells are able to discriminate between artificial sweeteners by subsequent TAS2Rs dependent signalling.

We speculate that, beside receptor-level, cellular level and neuronal coding, there might be a further variable in bitter stimuli discrimination, namely the signalling pathways.

Recombinant expression of the N-terminal domain of human T1R2 taste receptor: interaction with brazzein, a sweet-tasting protein

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Brazzein is a small (6.5 kDa) sweet-tasting protein originating from the fruit of *Pentadiplandra brazzeana* , a plant found in West Africa. Brazzein like all classes of sweet compounds is perceived through the activation of the T1R2/T1R3 heterodimeric sweet-taste receptor. T1R2 and T1R3 subunits are members of the small family of class C G-protein coupled receptors (GPCRs). Class C GPCRs possess a large N-terminal domain (NTD) linked to seven transmembrane domain by a cysteine rich domain (CRD). The NTD of T1R2 (T1R2-NTD) has been shown to contain the primary binding site for most of the sweet ligands. However, brazzein has been shown to require CRD of human T1R3 for receptor activation. In contrast, molecular modeling and docking studies have proposed that brazzein may interact primarily with T1R2-NTD and makes also favorable contacts with T1R3-NTD. To elucidate the contribution of T1R2-NTD to brazzein detection, we recombinantly expressed T1R2-NTD in *Escherichia coli* as a soluble cytoplasmic protein. Human T1R2-NTD was purified using three chromatography steps and characterized for its ability to interact with recombinant brazzein secreted by the yeast *Pichia pastoris*. Brazzein/T1R2-NTD interactions were measured using Bio-Layer Interferometry (BLI). This optical technique analyzes variations in the interference pattern generated from visible light reflected from an optical layer and a biolayer containing immobilized protein of interest. This recent method is powerful for studying protein-protein interactions and measuring both affinity constants and kinetic parameters. BLI experiments were performed first by immobilizing T1R2-NTD onto the biosensor and measuring brazzein binding. In a second experiment, brazzein was immobilized onto the biosensor and T1R2-NTD binding was followed using BLI. Both experiments demonstrated that T1R2-NTD binds brazzein with a K_d value of approximately 30 µM. This affinity is in agreement with the capacity of brazzein to activate T1R2/T1R3 receptor heterologously expressed in HEK cells and with sensory experiments conducted on humans. These data suggest that T1R2-NTD is the primary site for brazzein binding, which will be further investigated by site-directed mutagenesis conducted on brazzein and T1R2-NTD.

This work was supported by grants from the Regional Council of Burgundy France (PARI Agral 1) and the FEDER (European Funding for Regional Economical Development).

Abstracts of Posters Presentations

(Session 1)

Session 1	1st author	Title
1	Alevra Mihai	Ca2+ / BK channel clusters in olfactory neurons and odor coding
2	Amsellem Sherlley	The role of congruence in odor-taste interactions
3	Arzi Anat	Olfactory Aversive Conditioning During Sleep Reduces Addictive Behavior
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Ca2+ / BK channel clusters in olfactory neurons and odor coding

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Olfactory receptor neurons (ORNs) have high-voltage-gated Ca^{2+} channels whose physiological impact has remained enigmatic since the voltage-gated conductances in this cell type were first described in the 1980s. Here we show that in *Xenopus laevis* tadpoles these channels are clustered on the soma and co-expressed with BK channels. We found approximately five clusters per ORN and twelve Ca^{2+} channels per cluster. The action potential – triggered activation of BK channels accelerates the repolarization of action potentials and shortens interspike intervals during odor responses. This increases the sensitivity of individual ORNs to odorants. At the level of mitral cells of the olfactory bulb, odor qualities have been described to be coded by first-spike-latency patterns. The system of Ca^{2+} and BK channels in ORNs appears to be important for correct odor coding, because the blockage of BK channels not only affects ORN spiking patterns but also changes the latency pattern representation of odors in the olfactory bulb.

The Role of Congruence in Odor-Taste Interactions

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During the ingestion of food both, olfactory and gustatory percepts are elicited and bimodal olfactory-gustatory memories formed. Whether these distinct sensory events are perceived as one coherent entity likely shapes their impact on hedonic experiences and subsequent food choices. Prior experiences allow us to re-evaluate odor-taste combinations based on their perceived congruence or familiarity. For example, a sweet-sour orange juice is likely considered congruent, while a bitter orange juice would be considered as incongruent based on prior experiences with orange juice.

The effects of congruence on the perceptual experience of odor-taste combinations are still unknown and were aim of this study. We hypothesized that the degree of perceived concordance of the sensory inputs influences multisensory integration processes and thereby aspects of the perceptual experience, i.e. familiarity, pleasantness and intensity.

To test this hypothesis, we presented odor-taste pairs of varying degrees of congruence (100%, 75%, 50%, 25% and 0%) and collected subjective reports of intensity, pleasantness, familiarity and congruence using visual analog scales.

As expected, concordant stimulus pairs were perceived as more congruent then intermediate and incongruent pairs and *vice versa*. Moreover, familiarity and pleasantness were affected by congruence.

The results suggest that cross-modal olfactory-gustatory interactions are modulated by learned associations and perceived congruence between the sensory inputs.

Olfactory Aversive Conditioning During Sleep Reduces Addictive Behavior

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Cigarette smoking is an addictive behavior associated with significant morbidity and mortality. Efforts to treat this addiction using aversive conditioning have seen only limited success. The effectiveness of conditioning, however, may be greater when it is implicit rather than explicit. Given that learning during sleep is largely implicit, we set out to test the hypothesis that olfactory aversive conditioning during sleep will reduce smoking. A total of 76 smokers (26F, mean age = 28.7 ± 5.2 years) wanting to quit participated in the study. Subjects completed a daily smoking diary detailing the number of cigarettes smoked during seven days before and seven days after a one-day or night protocol of partial trace conditioning between cigarette odor and profoundly unpleasant odors (rotten fish and rotten eggs). We observed significant reductions in smoking following olfactory aversive conditioning during Stage 2, and rapid-eye-movement (REM) sleep but not following conditioning during wake ($F(2,31) = 3.66$, $p < 0.05$). Moreover, the reduction in smoking following conditioning during Stage 2 was greater and longer lasting in comparison to reductions following conditioning during REM (all $t(22) > 2.13$, $p < 0.05$). Finally, the reduction in smoking following conditioning during sleep was significantly greater than in two separate control sleep experiments that tested aversive odors alone, and the effects of cigarette odors and aversive odors without pairing. To conclude, a single night of olfactory aversive conditioning during sleep significantly decreased ensuing addictive behavior in a sleep stage dependent manner.

Odorant-binding protein engineering: impact on binding properties

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Odorant-binding proteins (OBP) are small soluble proteins present in millimolar concentrations in the nasal mucus covering the olfactory epithelium. OBPs belong to the lipocalin superfamily, whose members share a common scaffold made of 8-stranded β-barrel. This folding pattern defines a central polar cavity, named calyx, whose role is to bind hydrophobic molecules such as odorants. Although the physiological role of OBPs is not clearly understood, they are supposed to transport odorants from the air to olfactory receptors through the aqueous mucus. OBPs have been described in numerous species including pig, rat and human beings. Some animal species, such as pig, cow and human beings possess a single OBP, while others express distinct OBP subtypes, with rat and rabbit as examples. OBPs are broadly tuned and bind a large spectrum of volatile molecules. Interestingly, it has been shown that the three rat OBP subtypes (rOBP1, rOBP2, rOBP3) have different and complementary ligand profile, suggesting that OBPs are involved in odorant discrimination. Alignment of amino acid sequences of the three rat OBPs reveals the presence of an amino acid residue located in the binding pocket, which may be important for guiding binding specificity. Using site-directed mutagenesis, we generated mutants of rOBP3, in which this amino acid residue has been substituted by the others 19 amino acids. We tested the ability of wild-type rOBP3 and the mutants to bind 23 odorants belonging to different chemical classes using a fluorescent probe displacement. We observed that all the substitutions affected the binding properties of rOBP3. Interestingly, we found that some mutations decreased the affinity of rOBP3 towards some specific odorant molecules. In contrast, some substitutions generated OBPs possessing the ability to bind new odorant ligands with high affinities. Our work gives new elements to understand the binding mechanisms of OBPs and opens the way towards technological applications based on OBP, as odorant biosensors.

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The Time Course of Facial Expression Processing Modulation by the Olfactory Context: an ERP Study

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Detecting and understanding facial expressions are essential for human social adaptation. These skills are not independent from contextual effects, however, as environmental cues can enhance the comprehension of others' emotion. In particular, the odor context has been shown to modulate visual attention toward objects and faces. So far, little is known about the time course of olfacto-visual integration during facial expression perception. Using event-related potentials (ERPs), we studied the modulation of facial expression processing by hedonically-contrasted olfactory contexts. To keep visual attention constant along the experiment, a "go/no go" task was used in which adult participants (N=24) had to detect a red neutral face among several emotionally expressive faces. This task was run in 3 olfactory contexts: neutral (scentless air), pleasant (strawberry odor) and aversive (cheese odor).

ERPs showed that expressive faces enhanced the cerebral responses on several occipito-parietal electrodes. This effect started as soon as the P100 and lasted until the Early Posterior Negativity (EPN) that included the occipital P200 and N250. It consisted in the early differentiation of alerting expressions (disgust, fear, anger) from other expressions (sadness, happiness, neutrality) in the time window of the P100. In later stages (N170, EPN), the cerebral response clearly differentiated all expressive faces from neutral ones and, among expressive faces, disgust faces from other expressions. The olfactory context influenced the ERPs in response to facial expressions in two ways: 1/they enhanced the response to faces in the time window of the N170, but at central and left-lateral electrodes, regardless of the emotional content of expressions; 2/it differently modulated the EPN by increasing/decreasing the differential response to the various expressions.

The main finding here is the two-step influence of the olfactory context on facial expression processing. The first step (response enhancement to faces in both pleasant/unpleasant odor contexts) occurred in the time window of the N170. Several hypotheses may be proposed on the nature of these olfacto-visual interactions: i) odors may influence facial expression processing through the activation of facial motor areas that may mimick the perceived faces and feed-back on brain responses; ii) odors may modulate global attention or arousal in the observer; iii) both olfactory and visual inputs may activate common or interactive brain structures.

Brain areas involved in emotion regulation using olfactory stimulations: an fMRI study

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Emotion regulation has become a focus of interest since the past two decades and studies in cerebral imagery have recently highlighted its neural bases. However, the processes underlying the regulation of emotions when they are elicited by odorants remain unknown compared with other emotions inducer as visual stimulations. The aim of this study is to determine the brain areas involved in emotion regulation, using both pleasant and unpleasant odorants as inducer.

Eighteen subjects (10 males) were scanned during two sequences of 12 stimulations with either iso-amyl acetate (a banana-like odor, described as pleasant) or thioglycolic acid (rotten eggs odor, unpleasant), delivered with a custom-built olfactometer. For one sequence, subjects were instructed to naturally experience their emotion induced by odor inhalation (*Maintain*condition), and, for the other sequence, subjects were instructed to decrease the intensity of their emotion induced by odor (*Decrease* condition). The two sequences were counterbalanced between subjects.

Data were included in a General Linear Model to perform a random effect analysis. Paired Student t-tests (threshold: $p<0.005$ voxelwise, corrected with alpha=0.05) revealed strong activations in the left dorsolateral prefrontal cortex (dlPFC) and in the right anterior insula (dorsal part) for the "Decrease > *Maintain*" contrast. Moreover, Pearson tests shown strong negative correlations between activations of the left dlPFC and of the olfactory areas such as the right piriform cortex ($r=-0.78$, $p<0.000$) and the left ($r=-0.75$, $p<0.000$) or right ($r=-0.71$, $p<0.001$) medial orbitofrontal cortex (OFC). There was no difference between pleasant and unpleasant odors concerning the regulation task.

These results show that down-regulation in response to odors implies brain areas usually described for other emotion inducers such as the dlPFC, but also specific ones such as the anterior insula. Moreover, participants who most strongly increased their BOLD signal in the left dlPFC showed the lowest activations in the right temporal piriform cortex and in the medial OFC, which suggests a real top-down regulation on olfactory areas.

One broadly selective glomerulum in the olfactory bulb of Xenopus laevis tadpoles

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Calcium imaging experiments put high demands on temporal resolution and can only be evaluated reliably if at the same time the optical sectioning capability and the spatial resolution are sufficient.

For experiments in the olfactory bulb of *Xenopus laevis* tadpoles we developed an advanced line illumination microscope which does not only enable high frame rates in two channels but also provides improved contrast. Using this setup we investigated the β-glomerulus in the olfactory bulb of *Xenopus laevis* .

We found that the β-glomerulus does not only have a very salient position relative to the majority of pre-synaptic fibres but also responds to an unusual range of odorants. Responses to all 15 tested amino acids were observed. In a previous study of amino acid responsive glomeruli in the lateral cluster only 2 out of 67 were found responding to more than 11 amino acids and none responded to all 15 [Manzini *et al.*, 2007]. Furthermore, while most amino acid-responsive olfactory receptor neurons in *Xenopus laevis* have a cAMP-independent transduction pathway [Manzini & Schild, 2003], β surprisingly responds to forskolin which is an activator of the cAMP-dependent transduction pathway. Despite this, the β-glomerulus shows no response to any of the known cAMP-mediated odorants including alcohols, aldehydes and ketones.

With correlation analysis of responses to different amino acids and forskolin it was possible to show that apparently the whole structure reacts on each of the different stimuli. Thus β seems to be innervated by ORNs reacting already individually to all of the stimuli tested. Subsequent measurements with higher temporal and spatial resolution combined with activity correlation imaging made it nevertheless possible to distinguish different fibres.

By filling mitral cells with a calcium sensitive dye via bolus loading and a pre-synaptic counterstaining we could show that the β-glomerulus is connected to second order neurons and relays information to the respective mitral cells.

Spatio-temporal characterization of gustatory ERPs (gERPs) for the 5 basic taste qualities: sweet, salt, bitter, sour and umami

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Gustatory event related potentials (gERPs) can be used to investigate human taste function in a more objective way than psychophysical tests. One of the first who demonstrate this possibility was Kobal (1985). Recently, also other groups succeeded in the assessment of gERP (Hummel et al., 2010. Mizoguchi et al., 2002). However a standardized approach is still missing. Nowadays a new generation of taste stimulators (Gustometer, GU002/GM05, Burghart, Wedel, Germany) has been made available; it is specifically designed to elicit gERPs (Singh et al., 2011). For this reason in this work we aimed to characterize gERPs in response to the five basic taste qualities, using the gustometer for stimulus presentation. We intended to produce a standard protocol to be applied either in experimental research or in the diagnosis of gustatory dysfunction.

Thirty healthy, young volunteers attended the study (mean age: 23 years, s.d.=2 years). The experiment was subdivided in two sessions: (a) psychophysical intensity evaluation of the taste presented with the gustometer and (b) recording of the gustatory event related potentials (gERPs) using a 128-channel EEG system (BioSemi, Amsterdam, NL) .

The experiment provided (a) five calibration curves with stimulus concentration plotted against perceived intensity. From these curves the concentration corresponding to a medium intensity was chosen for the recording of gERPs (b). Following extensive pre-processing we were able to obtain responses to all five tastants. Further analyses will show whether the five tastants activate similar or different brain areas during the earliest stages of processing.

Neocortical markers in the olfactory cortex.

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Olfactory cortical regions such as the anterior olfactory nucleus (AON), piriform cortex (PC) and olfactory tubercle (OT) share many features with the cerebral cortex including clear lamination, an outer plexiform layer, pyramidal output neurons and similar interneuron types. How do their 2-3 layers compare with the six found in the neocortex? Substantial research has demonstrated that neocortical laminae can be described by a variety of developmental markers (e.g., Molyneaux et al, *Nature Rev, Neurosci.* 8, 2007). The present work examines the expression of some of these markers in the AON, the anterior PC and OT. Confocal images of coronal and horizontal sections were gathered for 5 markers that span the neocortex. **CART** , which has been reported to label neocortical layer 2 (NL 2), was found to be absent from the AON, but densely labeled cells in Layer 2 of the PC and OT. **CUX1,** reported in NL 2-4, was found in all portions of the pars principalis of the AON, but was differentially distributed within the structure. All of layer 2 in pars ventroposterior contained immunoreactive cells. However, profiles were observed in only the deep portion of layer 2 in pars lateralis and dorsalis and were very sparse in pars medialis. In the PC only Layer 3 was labeled and in the OT the marker was absent. **TRB1** , a marker for NL 2,3,5, and 6, was found throughout Layer 2 in the AON and in Layers 2 and 3 in the PC. It was absent from the OT. **Foxp2** (NL 6) was observed only in a line of cells just below the lateral olfactory tract in the AON. It was sparse in the PC but labeled Layer 2 in the OT. **Nurr1** (subplate) was absent from all regions. The findings indicate that the expression of these markers varies both within and between olfactory cortices, suggesting a) that understanding the developmental histories of the areas may yield important information about their similarities and differences and b) that the markers may be useful to distinguish between these higher olfactory system areas.

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Are Taste preferences related to submandibular endocrine or exocrine secretions ? A rodent study

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Context : Salivary gland secretions are involved in taste perceptions: EGF and NGF growth factors (endocrine activity) maintain taste buds and mouth epithelium integrity whereas salivary proteins (exocrine activity) are involved in the first step of bolus digestion (nutriment hydrolysis) but also in the pH and homeostasis controls for maintaining oral health. Nevertheless, the linkage between taste preferences and salivary protein secretion profiles is yet poorly informative. In this study, we raised the question about a correlation between salivary secretions and taste preferences by using a rat model.

Experiment: Taste preferences have been modulated in adult rats using endocrine disruptor exposures. Sweet, salt and fat preferences have been evaluated for three days before killing by using the two bottle choice method; salivary gland secretions have been evaluated by measuring the mRNA expressions of a relevant gustative proteins in submandibular. Correlation has been using Spearman test.

Results : By regarding mRNA expression of salivary protein gens, Gustine was significantly linked to fatiness ($p=0.03$) and to a lesser extent to saltiness ($p=0.055$) but not to sweetness preferences. Cystatin C is related to both salt and sweet preferences ($p<0.05$) whereas was KalliKreine 1 was correlated to sweet preference only ($p<0.05$). Amylase, lipase and mucin 10 expressions, as well as EGF and NGF endocrine secretions are not related to any taste preference in adult rat.

Conclusion: Taste preferences appeared to be related to saliva proteins resulting in exocrine but not endocrine secretions of salivary glands. These results suggested that saliva plasticity could reflect taste preference modulation and could be used to identify salivary biomarkers of taste preferences and feeding behavior. In this way, "omic" studies could be adequate tools for salivary protein screening.

In nose concentration of aroma compounds is modified by salivary protein composition and saliva stimulation

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Flavor is one of the most important organoleptic properties of food regarding consumer acceptability. The term flavor merges two senses, taste and smell. Volatile compounds (odor) are detected only by the smell, and savor only by the taste. During eating, saliva impregnates food and thus can impact the release of aroma compounds. On one hand, numerous studies have reported a variation of human salivary composition as a function of both individual and physiological conditions (rest or stimulated). On the other hand, it has been shown in model system that salivary proteins interact with aroma compounds inducing either a decrease or an increase in aroma release depending on both aroma compounds and proteins. However, the effect of saliva composition on retro-nasal aroma release during *in vivo* consumption has not been demonstrated yet.

Herein, we have performed an *in vivo* investigation on the release of two volatile compounds exhibiting contrasted hydrophobicity using atmospheric pressure chemical ionization – mass spectrometry. Ten subjects were recruited and their salivary composition has been characterized at rest and after stimulation (total protein and salivary lipocalin concentrations, alpha-amylase and lipase activity, salivary flux…). Subjects were asked to consume 3 mL of aromatized water solutions in three different physiological conditions: at rest, after stimulation by chewing a piece of parafilm and after elimination of the salivary film coating the mucosa by rinsing with water. The release curves were obtained from mass spectra and then different parameters were extracted such as maximal intensity, area under the curve, time of aroma persistence, maximal rate of release, time to reach 50% of the total release.

The results on salivary composition and salivary flux showed large variations between subjects and between rest and stimulated conditions. Aroma release exhibits variation as a function of subject, condition of consumption and hydrophobicity of aroma. More particularly, the time of aroma persistence increased with aroma hydrophobicity and was significantly lower after mouth rinsing. Moreover, it was positively correlated with the concentration in lipocalin. This protein, which is produced by the van Ebner gland, belongs to the odorant binding protein family.

A transient receptor potential is expressed with multiple splicing isoforms in the Codling Moth Cydia pomonella

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Transient receptor potential (TRP) channels are an ancient family of cation channels that have been found in many eukaryotic organisms, from yeast to human. Since the first identification of a TRP in *Drosophila melanogaster* , several TRPs have been identified and functionally characterized in other insect species.

TRPs are involved in the reception of physical and chemical stimuli but also of metabotropic triggers, mediating membrane depolarization in response to environmental stimuli. Regulation of TRPs at multiple levels, including transcription, mRNA splicing, protein synthesis/processing in ER and Golgi apparatus as well as membrane trafficking, and post-translational modifications have been reported. It is speculated that different TRP splice forms serve various distinct functional roles.

Identification of TRP candidates in insect pests and understanding their functional role in sensing environmental cues of different modalities is intriguing and may open for novel applications in agriculture. For instance, TRP-active compounds reported to have somatosensory properties were found to be active on insect pests at the electrophysiological and behavioral level.

Using next generation sequencing of the antennal transcriptome, we have identified five candidate TRPs in the fruit pest *Cydia pomonella* (L.). A phylogenetic analysis revealed that candidate antennal TRPs belong to the TRPA, TRPC and TRPV sub-families, known to be involved in various sensory modalities such as noxious heat avoidance, hygrosensation and phototransduction in *D. melanogaster* . The full-length sequence encoding one of these five TRPs was obtained by RACE-PCR, and its expression pattern was investigated in different body parts of adult moths, in both males and females. The existence of multiple splicing isoforms of this candidate TRP was demonstrated by PCR, using primers crossing regions of alternative splicing. Interestingly, while some isoforms were expressed in all body parts, others were differentially expressed, depending on sex and body parts.

Odours, can they impact on salivation: Myth or scientific evidence?

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Nowadays, questionnaires are largely used to directly measure eating behaviour. However, the responses obtained through questionnaires may suffer of a "social desirability bias" resulting in responses more in line with social norms than reflecting the true opinions of individuals. To overcome this bias, some physiological measures, such as salivation, can be interesting objective indicators. Salivation can be modulated by different stimuli and various studies have shown that odours can impact salivation (Spence, 2011, Mattes, 2000) and can impact on food choices (Gaillet et al., 2013; 2014).
Objectives of the present research are to determine if different kinds of odours can increase total salivation and responses obtained via direct questionnaires (for example, hedonic rating, perception, …) can be linked to salivary flow.
Thirty men with good health status, between 18 and 45 years old, were recruited to participate to three one-hour sessions. They were asked to smell five flasks containing four different odours: lemon, strawberry, bacon, thyme and a control flask without odour. They had to smell each odour during two minutes and had to make a five-minute break before smelling the following one. After smelling one odour, each participant had to spit saliva accumulated in his mouth during the 2min of smelling in a corresponding vial. Vials were weighed before and after each session to determine salivation. After saliva collection, participants were asked to fill in a questionnaire. For each odour, they rated hedonic score, odour intensity, odour appetence and their salivation feelings. Moreover, they were asked to identify each odour.
Analysis of variance (ANOVA) and Newman-Keuls statistical tests were carried out. First of all, results showed an impact of odours on salivation compared to control flask without odour ($p<0.0001$). Lemon and ham odours induced the highest saliva production. Nevertheless, important inter-individual variations were observed. No correlation was observed between responses obtained to the questionnaire and salivation.
The main result of our study emphasizes that different types of odours can really increase salivation. However, objective measure of the salivation cannot be linked to the declarative responses obtained via questionnaire. Such result highlights a classical discrepancy observed between declarative data and objective measures in the consumer behaviour domain. In future research, it will be worth to conduct other experiments to study odour effects on salivary flow of particular salivary glands to obtain more precise measures concerning the timing and quantity.

Impact of an olfactory and auditory priming on the attraction towards foods with high energy density

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Recent research in food science suggests that unconscious processes may influence a significant part of consumers' food choices. Studies of Gaillet and collaborators (Gaillet et al., 2013; 2014) demonstrated that non-attentively perceived fruity odours impact food choice, guiding participants toward items containing more fruit and/or vegetables. The first objective is to determine if another kind of odour, such as a sweet-fatty odour, could increase food choices towards high energy density foods. The second objective is to compare the effect of an olfactory cue to the effect of an auditory nutritional message.

147 participants took part in the experiment, and were assigned in four different conditions: a control condition (n=37), a scented condition (n=38), an auditory condition (n=37) or an auditory-scented condition (n=35). All participants remained in the waiting room for precisely 15 min, during which they performed a 'lure' task, consisting of completing a questionnaire on communication skills and representational systems. For the scented condition, the participants were unobtrusively exposed to a "pain au chocolat" odour. For the auditory condition, they were exposed to an audiotape composed with radio podcast and nutritional message ("for your health, avoid to eat too much fat, too salty, too sweet"). A third group of participants were exposed to both olfactory and auditory primes in the same time. In the control condition, no stimulation was delivered. Following this waiting period, all participants moved into a non-odorised test room where they were asked to choose, from dishes served buffet-style, the starter, main course and dessert that they would actually eat for lunch.

Results showed that participants primed with odour of "pain au chocolat" tended to choose more desserts with high energy density (i.e., the waffle) than participants in the control condition ($p=0.06$). Unexpectedly, participants primed with nutritional auditory message chose to consume more desserts with high energy density than participants in the control condition ($p=0.03$). In the last condition (odour and nutritional message), participants chose to consume more desserts with high energy density than participants of the control condition ($p=0.01$) revealing an additive effect of the two primes.

To conclude, our results are in line with previous research of Gaillet at al. (2013; 2014), and confirm that a non-attentively perceived odour could impact food choice. This impact applies on food choices of high energy density foods. Surprisingly, auditory priming with nutritional messages guides individuals towards more dessert with high energy, despite the fact that participants were note able to recall the message. Such a result queries about the effectiveness of public health messages when they are broadcast on radio.

Role of CYP6d5 in the metabolism and the sensory perception of caffeine in Drosophila melanogaster

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The detoxification and elimination of potentially harmful chemicals are critical processes for most organisms. Cytochromes P450 (CYP) can take in charge potentially toxic molecules, biotransform and eliminate them outside the organism. CYP are highly expressed in tissues involved in detoxification processes but also in sensory organs suggesting their implication in the modulation of chemoperceptive processes. They likely neutralize the stimulus molecule after its detection, avoiding the saturation of receptors and allowing the neuron to quickly respond to another stimulus.

The aim of our study consisted in investigate the role of CYP6d5 in the metabolism and the sensory perception of caffeine in *Drosophila melanogaster* .

Using a transcriptomic study on adult male flies exposed to caffeine, we identified the candidate CYP6d5 whose expression was up-regulated in whole body and sensory appendages by caffeine. Since enzymes, whose expression is modulated by a xenobiotic, generally participate in its degradation, our results suggest that CYP6d5 could be involved in caffeine metabolism.

For the first time, we analysed caffeine metabolism in drosophila and we concluded that it is CYP dependent as in vertebrate.

We hypothesized that a modulation of CYP6d5 expression should trigger a modification of caffeine (i) metabolism and (ii) perception. A knockdown of CYP6d5 in whole body cells led to the disappearance of theobromine, one of the major caffeine metabolite, attesting a specific role of CYP6d5 in caffeine metabolism into theobromine.

Moreover, the expression pattern of CYP6d5 in the gustatory organs revealed a diffuse expression in the sensory pockets at the base of the sensilla, both in neurons and accessory cells. Furthermore, we tested the ability of transgenic flies presenting a modulation of CYP6d5 expression in different subpopulations of sensory cells to detect caffeine using MultiCAFE behavioural test. A significant alteration of the detection of the caffeine was observed when CYP6d5 expression is down regulated in chemosensory neurons and more specifically in the Gr66a neurons, suggesting a role of CYP6d5 in detection mechanisms of caffeine.

This is the first characterization of caffeine metabolism in *Drosophila melanogaster* . We also involved for the first time a CYP in gustatory perception. The critical role of CYP6d5 in caffeine metabolism was correlated with its significant impact on caffeine detection.

Context independent memory for odors and words

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Context-dependent memory (CDM) refers to the effect of enhanced memory performance when the context during encoding is reinstated at testing as compared to testing in a different context. Previous research has shown that verbal memory is better when encoding and retrieval occur in the same as compared to different contexts (Smith & Vela, 2001). However, to the best of the author´s knowledge CDM effects on odor memory has not yet been studied. In a within-subjects design, 63 participants (34 women) encoded 12 odors and 30 words. Two rooms were used and their main difference was the color of the light. Recognition memory for odors and words was assessed both in the same and in a different room from where encoding occurred. For odors, the participants attempted to identify each stimulus and evaluated them for familiarity, intensity, and pleasantness. Word recall was tested at one occasion. The results showed no statistically significant effects of context change on recognition (d´) for either odors (M same = 1.51, *SD* = .63; *M* different = 1.43, *SD* = .60) or words (M same = 1.60, *SD* = .74; *M* different = 1.48, *SD* = .83). Moreover, recall of target words did not differ significantly between the same (M = .28, *SD* = .10) and different contexts (M = .26, *SD* = .11). In sum, the results indicate that neither memory for odors nor words is dependent on being tested in the same physical environment as during encoding. These null findings might be due to the rather small contextual manipulation (cf. Fernandez & Glenberg, 1985). Future studies should investigate the effects of more relevant contextual cues on odor memory.

Pyrophosphates and Cat food palatability

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Because domestic cats are extremely sensitive to the palatability of their foods, it is necessary to understand the mechanisms underlying cat preferences. Food preferences may be influenced by several factors including nutritional needs, sensorial characteristics, as well as previous feeding experience. Pyrophosphate salts are well known ingredients used in cat food. We sought to understand their mode of action by two different approaches: a behavioral study of the effect of previous feeding experience and a mechanistic experiment to search for specific taste receptors.

In order to investigate the influence of cats' exposure to pyrophosphate on taste preference, 3 groups of queens were bred on 3 different diets: a control diet containing no pyrophosphate (CT), a diet containing trisodium pyrophosphate (PP) and a diet containing monosodium glutamate (MG).

At weaning, the kittens born to queens receiving either the PP or MG diet were further separated in 2 groups, one group staying on the gestational diet, the other group moving to the CT diet. After a qualification phase for palatability testing, we evaluated the initial preference of the kittens for the 3 different diets (CT, PP, MG) using a standard two-pan test.

The molecular and biochemical study was based on the heterologous expression of the umami feline taste receptor (T1R1/T1R3) transferred into human embryonic kidney cell line (HEK 293). This cell line was transfected with the polynucleotides encoding the feline T1R1/T1R3 receptor and the G protein, G-α 15. The cells containing T1R1/T1R3 receptors were loaded with a sensitive calcium indicator dye that measures intracellular calcium concentration. The activation of the receptors in the presence of different tasty solutions (amino acids, PP alone or association of both) was measured using fluorescence ratiometric determination of intracellular calcium.

This study allowed us to establish the unique, immediate and robust preference of cats for pyrophosphate. The feline receptor T1R1/T1R3 is involved in this perception and preference. It is also hypothesized pyrophosphate act as a modulator of the activity of the amino acid receptor T1R1-T1R3. The developed method can allow the screening of different compounds and the determination of their potential as cat palatability agents.

Olfactory Marker Protein and olfactory transduction: a puzzling case

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Olfactory marker protein (OMP) is a small cytosolic protein expressed in mature olfactory receptor neurons (ORNs). Since its discovery over 30 years ago its mechanistic contribution to olfactory transduction remains in large parts unclear. Previous studies have shown OMP involvement in the ORNs odor response and cAMP kinetics by a yet unknown mechanism. Because basal cAMP levels are dictated by the constitutive activity of the olfactory receptor (OR) we sought to investigate how OMP interact with elements of the cAMP signaling inside the cilia of the ORNs. We began to address the mechanisms of OMP interactions in an OR-specific manner by generating two OMP KO lines that also expressed GFP with either the low basal activity mOR-EG OR or the higher basal activity M71 OR. This difference in basal OR activity translates into low and high basal action potential firing in mOR-EG and M71 ORNs respectively. Striking changes occurred in the OMP KO. The lack of OMP greatly prolongs the odorant response independent of expressed OR but greatly reduced the variance or noise levels in a OR-dependent manner for M71 ORNs. This was accompanied by a large reduction in basal action potential firing in M71 OMP KO ORNs. In general, in the absence of OMP mOR-EG and M71 ORNs have essentially indistinguishable basal firing rates. This demonstrates that OMP plays a major role in maintaining appropriate basal activity with possibly important consequences for olfactory coding.

I've got your nose, I know how you feel: odor effects on the visual processing of faces in 7 month-old infants

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The first year of life is critical for the development of the abilities to process facial expressions. Infants learn then to discriminate and categorize the different facial expressions based on visual inputs. Nevertheless, our perceptual integration of the environment is based on multisensory processing since birth, leading to five senses representations and reciprocal influences between them. Thus, the inputs from the other senses do also influence how facial expressions are processed by infants. For example, audition is known to be influential on the integration of emotion from facial expressions. Here, we focus on the potential role of olfaction in the early processing of facial expressions. It is well-known that hedonically-contrasted odors trigger consistently distinct facial expressions in infants and young children. Thus, infants should progressively form links between their own odor-induced facial patterns and those they see in their caregivers and multiple interactants. We hypothesize that infants might manifest some cognitive expectancies about the particular facial configuration that a face *should* adopt when they are exposed to an odor conveying a given hedonic valence. This type of odor-vision interactions has been repeatedly reported among adults (e.g., Leppänen & Hietanen, 2003).

The present study aimed to investigate whether hedonically-contrasted olfactory contexts can modulate the visual exploration of faces in 7-month-olds. An habituation paradigm was used. The habituation phase consisted in 6 infant-controlled presentations of a neutral female face on a screen without any odor. The test phase consisted in 4 trials presenting the same neutral face, but with or without a given odor context. The odor context was created by the diffusion of an a priori positive odor (strawberry), an a priori negative odor (butyric acid) and a scentless control context. The effect of the odor context on the infants' visual exploration of the face was measured directly using eye tracking. It came out that the odor exposure lead to a global increase of the time infants spend looking at the face, and that this increase is focused to particular areas of the face on which action units are to appear on average. These results indicate the early operation of olfactory-visual integration of facial expressions in 7-month old infants.

A new electrogustometer for taste sensitivity measurements

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Electrogustometry was already known in the times of Volta (1745-1827) and further developed in the 1940ies (Bujas, 1936) as a method for evaluating taste sensitivity and its deficits as documented in the literature. With an anodal stimulation, a minute current moves the cations of the subject's saliva toward its receptors (iontophoresis) including ENaCs signaling Na^+ and H^+. A taste threshold can be determined, currents being low enough not to stimulate trigeminal nerve endings which results in a recognized somato-sensory sensation. However, it is also possible to reverse the current and stimulate with anions, dipping the subject's tongue in infra-threshold concentrations of sapid ionized stimuli (e.g.: saccharin, cyclamate, acesulfam-K^{TM}), which elicits sweet taste. Moreover, electrophysiological studies with iontophoretical application of cations and anions in rodents also results in taste nerve (CT) responses.

We have developed a digital, portable electrogustometer, with the French start-up "Myrobotics". A 7.5 x 6 x 2.5 cm electronic module including a constant current generator can be Bluetooth controlled by a tablet or a smartphone. We have implemented three protocols: (1) the standard one which is a free monadic presentation, (2) a protocol using the staircase presentation of Dixon which calculates a P_{50} (threshold) using the binomial law, and (3) a third one associating forced-choice pair comparison (stimulus vs no stimulus), the Dixon staircase protocol and the calculation of P_{50} thresholds. This later one allows double blind experiment. A single assessment of threshold needs about 3mn.

The stimulating electrode is either a sphere 10mm o.d. or a disk 8mm diameter mounted on a spring to ensure a contact of the total surface without pressure. Such an electrode improves the reproducibility from one experimenter to another one.

Data compare the results obtained with the three protocols, with both electrodes and different experimenters.

Applications are targeting evaluation of sensitivity (both deficit and recovery) in health and disease, in medicated subjects, during and after chemotherapy, before and after stapedectomy, dental desafferentations, smoking cessation, in prevention programs in adults and children, and for the selection of panel subjects in the Food industry.

Coll.: Eberhardt, A., MyRobotics, Puteaux, France ; Tiercelin, JY., Parra, P., service prototypage, CNRS, Gif sur Yvette, France.

The strange case of the chondrichthyan olfactory system

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The main olfactory system has been identified in all the classes of vertebrates, while the vomeronasal system is lacking in some groups, such as birds and primates, which presumably have lost it. Chondrichthyes are one of seven vertebrate classes. The peripheral olfactory organs of these fishes are large olfactory rosettes, with a huge sensory surface, which does not bear sensory cilia, a unique condition in all studied vertebrates (Ferrando and Gallus, 2013). Only the genome of one species, elephant shark *Callorhinchus milii,* has been sequenced to date and only 3 genes for olfactory receptor (ORs) and 2 genes for trace amine-associated receptors (TAARs) were found (Venkatesh et al., 2014). However, 1 gene belonging to the vomeronasal receptor family of type 1 (V1Rs) and 32 genes of type 2 (V2Rs) have been identified in *C. milii* . We applied immunohistochemical methods on the peripheral olfactory organ of different Chondrichthyes in order to detect the presence and distribution of G protein alpha subunits, usually coupled to ORs and V2Rs. The immunohistochemical detection of G protein α subunits in the olfactory organ of the elasmobranch *Scyliorhinus canicula, Etmopterus spinax, Raja clavata, Raja asterias, Torpedo marmorata* and the holocephalan *Chimaera monstrosa,* did not reveal the presence of Gαolf, highlighting the presence of Gαo and/or Gαi in virtually all sensory neurons. Moreover, as the gene for Gαolf is present in the *C. milii*genome, we investigate the expression of mRNA for Gαolf in the olfactory rosette of *C. milii* , *C. monstrosa* and *S. canicula* , and we found it is not expressed.
From our data and from the literature, we can suggest that Chondrichthyes presents a very particular olfactory system, compared to that of all the other vertebrates, showing almost exclusively vomeronasal features. Further investigations could lead to interesting acquisitions on the biology of these fishes and on the evolution of chemoreception in vertebrates.

Deciphering the odorant binding protein – olfactory receptor interactions

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Olfactory receptors (OR) belong to the family of GPCR and participate to the recognition of odorants. Prior to the activation of the OR, odorants are disolved in the olfactory mucus by odorant binding proteins (OBP).[1] Belonging to the family of lipocalins, they are small carrier proteins and are considered as non-specific binders.[2] OBP would contribute to the olfaction process by carrying hydrophobic odorant molecules to the OR. Due to the transmembrane nature of these receptors, it remains a hard task to obtain their crystal structure. Molecular modeling methods are perfectly suited to provide relevant three-dimensional models of unknown structures to further gain insights on the structural features of protein-protein complexes.

Molecular models of hOR2T4 and rORI7 have been built using both *ab initio* [3] homology modeling approaches. To understand how OBPs release odorants and participate to the modulation of OR activation, state of the art protein-protein docking [4] have been performed to predict OR-OBP complexes structure (Figure 1). We propose models of interactions showing how rOBP3 is connected to the extracellular loop 2 of hORI7. To decipher the underlying molecular mechanism, molecular dynamics simulations have been carried out to estimate the octanal-rOBP3 free energy of binding. The formation of the OBP-OR complex destabilizes the odorant-OBP interactions prior to the release of the odorant.

Olfactory sensitivity in elderly with Parkinson's disease

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OBJECTIVES: Hyposmia is an interesting and a most common pathological characteristic of Parkinson's disease [Doty, 2013; Foguem and al, 2014] and is considered as a PD marker mainly at the early stage of the PD, in younger patients (< 65 years). Because few studies have considered olfactory loss in elderly patients with PD, the aim of this study was to assess olfactory function of elderly patients with idiopathic PD using odour detection thresholds compared to controls paired by age.

***METHODS*:** Olfactory detection thresholds of Phenyl Ethyl Alcohol (PEA) [activating the olfactory system] and n-Butanol (BUT) [activating both olfactory and trigeminal systems], were determined in ninety-two patients with PD aged over 65 years old [mean age: 74.8 +/- 8.8 years, range: 65-93 years] and in ninety-two healthy controls matched for age and gender [mean age: 79.8 +/- 8.8 years, range: 65- 90 years]. The study also included neuropsychological evaluations and stage of PD estimations.

RESULTS: The results show significant impaired olfactory (CN I) detection sensitivity in relation to PEA thresholds in patients with PD compared to controls independently of age and stage of PD. There was also significant impaired detection BUT thresholds. The differential value between mean PEA dilution thresholds (controls – PD) was greater than the differential value between mean BUT dilution threshold (controls – PD). PEA and BUT thresholds were significantly correlated in both patients with PD and controls.

***CONCLUSION*:** The findings of this study show that olfactory sensibility deficits mediated by PEA and BUT observed in young patients (<65 years) with PD are also observed in older patients (> 65 years) compared to controls paired in age.

Caffeine metabolism in Drosophila melanogaster.

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Caffeine (1,3,7-trimethylxanthine), an alkaloid produced by plants, induces various physiological effects on organism and can also act as a potent natural insecticide.
In vertebrates, the metabolites derived of caffeine have been identified, and the metabolism characterized. Caffeine is metabolized by cytochrome P450 (CYP), in four main dimethylxanthines metabolites. CYPs belong to the metabolism enzyme network in charge of the elimination of xenobiotics from the organism. However, the caffeine metabolism of insects remains unknown.

In this study, we separated 8 caffeine metabolites from male *Drosophila melanogaster* fed with radiolabelled caffeine. Among the major metabolites: theobromine, paraxanthine and theophylline were identified and theobromine appeared as the highest synthesized metabolite.

A transcriptomic screen of *Drosophila* exposed to caffeine revealed the coordinated variation of a large set of xenobiotic metabolizing enzyme genes including several CYPs highly overexpressed. Flies treated with metyrapone, an inhibitor of CYPs, showed a dramatically decreased of caffeine metabolism, indicating the involvement of CYPs in this process.
This is the first approach of caffeine metabolism in insects. It opens a large number of perspectives regarding the physiological role of the metabolites or the use of theobromine as a biomarker of CYP activities in *Drosophila*.

Urea effects on the olfactory epithelium of developing Danio rerio

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Aim of this study was to evaluate the effects of urea on the molecules involved in olfactory signal reception and transduction, and on nitric oxide synthase (NOS) in the olfactory epithelium of vertebrates. The study is based on evolutionary and clinical clues. The physiologically uremic fishes (the Chondrichthyes class) lack olfactory cilia, olfactory receptors and the G protein alpha subunit olfactory-type ($G\alpha_{olf}$) (Ferrando and Gallus, 2013). Chronic renal diseases cause olfactory impairment through an unknown mechanism (Landis et al., 2011).

Danio rerio embryos were exposed to 75 mM urea concentration from 24 to 96 hours post fertilization. The endocytosis of Neutral Red dye in the olfactory mucosa was detected in control and urea-exposed larvae as a marker of binding and internalization of the Olfactory Receptors. The presence and distribution of $G\alpha_{olf}$ were investigated in the olfactory epithelium of control and urea-exposed larvae, using a commercial antibody. Nitric oxide (NO) has important roles in the olfactory epithelium, meanwhile urea is known to affect its production. Thus we evaluated, in control and urea-treated larvae, the presence of NOS in the olfactory epithelium using immunohistochemistry and a histoenzymatic reaction. We also exposed *D. rerio*larvae to a NO-production inhibitor, (NG-L-Nitro-Arginine), and a NO donor (Nitroprusside) comparing them to the control and urea-treated fishes.

The Neutral Red internalization, and possibly the olfactory receptors presence and functions, was unaffected by urea. Both $G\alpha_{olf}$ and NOS were increased in urea-treated fishes than in control. The treatment with NO-production inhibitor and NO donor gave interesting clues allowing some considerations about the role of NO in the olfactory transduction molecules. Further investigations are needed in order to understand the actual effects of urea on the olfactory system of pathologically and physiologically uremic vertebrates.

Altered perirhinal cortex activity patterns during recognition memory in aged rats

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The perirhinal cortex (PRh) is one of the most heavily damaged cortical areas in Alzheimer's disease (AD) and the focus for disease onset (Van Hoessen, 2000). PRh pathology is associated with chemosensory identification dysfunction which represents the early sign of Alzheimer's disease. Preclinical odorant identification deficits precede other sensory modalities of recognition memory impairment (Aliani *et al.*, 2013). Given the fact that a similar dysfunction often appears in healthy aging, we have atempted to dissociate age and disease related changes in PRh function. The impact of normal aging on PRh activity was assessed during flavor recognition memory using c-Fos immunoreactivity as a marker for neuronal activity. Adult (3-month-old) and aged (24-month-old) Wistar male rats were exposed to a vinegar solution on a daily basis for a period of six days. Consistent with previous results (Gallagher and Burwell, 1989; Misanin *et al.*, 1985; Morón and Gallo, 2007; Pelleymounter and Cullen, 1993) aged rats showed slower attenuation of neophobia, thus indicating deficits of flavor recognition memory. An opposite pattern of PRh activity was found in adult and aged rats as the flavor became familiar. Whilst adult rats exhibited a higher number of PRh c-Fos-positive neurons during the presentation of the novel flavor than during the second and sixth presentation, in aged rats the number of PRh c-Fos-positive neurons was higher during the presentation of the familiar flavor in the last session than in the first and second. The most relevant finding is the opposite activity pattern during the sessions, suggesting a different role of the area depending on the age. Thus, PRh seems to be involved in processing novelty in adult rats but familiarity in aged rats. Similar results to those found in aged rats have been previously reported in younger 8-month-old rats subjected to surgery (Gómez-Chacón *et al.*, 2012). It is conceivable that aging might induce a reorganization of the brain circuit involved in recognition memory. The results suggest that the role of the area changes during aging and can help to dissociate PRh dysfuntions induced by neurodegenerative diseases and normal aging.

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Componental Identification of Odors in Mixtures

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When chemicals with as few as four different odors are presented together, humans cannot recognize any individual odor. In higher order mixtures with equally intense components, none can be identified. Recent research on selective adaptation of mixtures discovered odors of individual mixture components emerge after prior presentation of all other mixture constituents for a few seconds. This rapid improvement in component identification, mimicking a rapid intensity increment, relates directly to how olfactory qualities are coded in humans. Previously tested compounds with some common chemical features showed olfactory receptors do not focus on isolated molecular features. Rather, they likely recognize entire molecules associated with perceived olfactory qualities (notes). Rapid selective adaptation also unmasked each component in mixtures of the chemicals (*odors*) benzaldehyde (*cherry*), maltol (*caramel*), guaiacol (*smoke*) and methyl anthranilate (*grape*). Identification of mixture components was nearly double for a fresh extra component compared to lingering ambient components. This occurred despite the domination of more intense benzaldehyde and identification of methyl anthranilate as *smoke* as well as *grape*. Methyl anthranilate may activate two or more olfactory receptors, one of which also detects guaiacol. Methyl anthranilate and guaiacol are ortho-disubstituted benzenes of comparable size with related functional groups (methyl ester vs. methyl ether, amino vs. hydroxyl). Thus, selective adaptation parses characteristic odors of stimuli that may be weak or strong or have multiple notes. Central suppression among mixture components and peripheral selective adaptation of each component, intensity adjustments achieved through the olfactory system, determine which notes are perceived at any point in time. Latest (un-adapted) and greatest (most intense) odor notes dominate. Likely, the stereotyped olfactory pathways accommodate an *olfactory receptor rivalry*, reducing the number of signaling receptors to the few that are most active. Each receptor variant, responsive to a range of ligands, is expressed in a set of olfactory sensory neurons that project all axons to a few olfactory bulb glomeruli. The olfactory bulb and olfactory piriform cortex refine odor discrimination downstream by modifying signals via inhibition. Thus, dynamic selective adaptation of stimulus components suppressed in mixtures reveals *the combinatorial code for odor is componental*, containing discrete notes. Eighty-thousand pied binary combinations are obtainable from four hundred receptors for componental identification of odors in mixtures.

Landscapes of taste

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Electronic noses/tongues (eN/eT) comprising of an array of non-specific and low-selective sensors have emerged as promising tools for analysis of complex mixtures in gas or in liquid in various domains. For construction of eN/eT with high cross-sensitivity, one of the main challenges is related to the design and production of sensing materials capable of making differentiation even among similar species. We have recently developed a combinatorial approach that simplifies greatly preparation of sensing receptors.[1] In this concept, an array of differential receptors was prepared by mixing at well-controlled proportions and self-assembly of a restricted number of building blocks, which are small and easily accessible molecules with different physicochemical properties. Using an optical detection system such as surface plasmon resonance imaging, an electronic tongue was constructed, which generated continuous recognition patterns including 2D continuous evolution profile and 3D continuous evolution landscape for samples in liquid. The obtained electronic tongue is versatile. It is efficient not only for protein analysis such as identification and differentiation of different proteins [2] even among the same family [1] at a nanomolar concentration but also for analysis of complex mixtures such as discrimination of different milk samples (plant-based and animal-based) [3] and monitoring deterioration of UHT milk. Importantly, the eT has good measurement-to-measurement and batch-to-batch reproducibility and long-term stability, capable of operating after a minimum storage period of 5 months.

Glibenclamide induces CCK secretion via TRPA1 activation in STC-1 cells

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Glibenclamide enhances insulin secretion by blocking ATP-dependent potassium channels in pancreatic beta-cells and is widely used in the treatment of type 2 diabetes mellitus. It induces adverse effects, among which are abdominal pain and gastrointestinal disturbances. Gut hormones mediate nutrient and non-nutrient signals from the gut to target tissues that regulate gastrointestinal functions, including secretion, motility, digestion, and absorption. One of the gut hormones, cholecystokinin (CCK), is produced in enteroendocrine cells dubbed I cells. The expression of transient receptor potential ankyrin 1 (TRPA1) channels has been demonstrated in human and mouse duodenal mucosa and that activation of TRPA1 stimulates CCK secretion in enteroendocrine STC-1 cells. This study investigated that glibenclamide can induce CCK secretion from STC-1 cells, and then examined the mechanisms underlying the CCK secretion. Glibenclamide induced CCK secretion with an EC_{50} of 0.10 mM when the secretion of 0.10 mM allyl isothiocyanate was regarded as maximum. Glibenclamide increased intracellular calcium concentrations in the cells. CCK secretion and intracellular calcium increase induced by glibenclamide was abolished in the absence of extracellular calcium. In addition, the inhibition of TRPA1 suppressed glibenclamide-induced CCK secretion and intracellular calcium increase. The results demonstrate that glibenclamide induces CCK secretion via TRPA1 activation in STC-1 cells, which may explain some of the adverse effects of the drug.

The chemosensory transcriptome of the cotton leafworm Spodoptera littoralis

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The sense of smell is determinant for vital insect behaviours, including mate and food seeking, oviposition and predator avoidance. To decipher the underlying mechanisms in the cotton leafworm, *Spodoptera littoralis*, we sequenced its chemosensory transcriptome. Among the ~ 77000 annotated expressed contigs, we described large repertoires of candidate odorant-binding and chemosensory proteins, ionotropic receptors and olfactory receptors, and candidate odorant-degrading enzymes. Comparison between adults and larvae revealed different but somewhat overlapping expression of the chemosensory genes in the different developmental stages.

The transcriptome was also used to investigate the transcriptional changes induced by 24h starvation in the chemosensory tissues of *S. littoralis* caterpillars, using RNAseq expression profiling. Among the transcripts regulated upon starvation, we identified genes potentially involved in olfaction.

These approaches establish the use of transcriptomic sequencing for the identification of divergent sequences such as those encoding chemosensory receptors in a species for which no genomic data are available, and for investigating olfactory plasticity via digital gene-expression profiling.

Is there a link between perceived flavor intensity and food intake?

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Many studies have dealt with the effect of sensory perception of foods on appetite and food intake. In this field, research has mainly focused on the influence of food palatability and on sensory properties of food involved in sensory-specific satiety. In contrast, few studies have investigated the link between flavor intensity and food intake. Thus, the present study compared the intake of two types of smoke-cured sausages (i.e. *Morteau* sausage, one "low-smoked" and one "extra-smoked") which supposedly differ in the level of perceived flavor intensity.

Subjects (N=62; mean age = 22 years±2) were recruited among students of the University of Franche-Comté and were divided into two groups according to the type of *Morteau* sausage (i.e. "low-smoked" and "extra-smoked"). First, subjects were presented with ten pieces of sausage (total 50g±1g) and were asked to rate on a scale from 0 (absent) to 10 (extremely present) the perceived intensity of ten flavors considered as the most prominent by experts in *Morteau* sausage evaluation (e.g. mushroom, cabbage, smoke flavor…). Second, subjects were given small pieces of sausage (total 70g). They were asked to complete a questionnaire on their eating habits (to shift their attention from the aim of the experiment) and allowed to eat as much or as little as they wanted. Food intake was measured by calculating the amount of sausage consumed.

Results showed that the perceived flavor intensity was higher in the "low-smoked" group (constituting the "high-intensity" group) than in the "extra-smoked" group (constituting the "weak-intensity" group). Interestingly, findings revealed that subjects from the "high-intensity" group ate significantly less than subjects from the "weak-intensity" group.

This study suggests the existence of a link between the level of perceived flavor intensity and food intake which strengthens the importance of sensory processes in meal termination and satiety.

Activity of neural reward circuits in response to food odors: an fMRI study of liking and wanting

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Brain reward circuitry mediate food cued liking and wanting responses. Here, we explore the differential involvement of some neural structures (ventral and dorsal striatopallidal area, orbitofrontal cortex (OFC), anterior insula and anterior cingulate cortex (ACC)) in these two psychological components of reward. Using event-related functional magnetic resonance imaging (fMRI), we have asked 12 healthy female participants (mean ± SD: 24.14 ± 3.06 years) to rate pleasantness of food and non-food odors (liking) and the desire to eat the odor-evoked food (wanting) in both hunger and satiety states. Activities in the structures of interest mentioned above were contrasted in function of task (liking vs. wanting), odor category (food vs. non-food for liking task), and metabolic/motivational state (hunger vs. satiety).

We found that the activity in nucleus accumbens and ventral pallidum was different in liking or wanting tasks in function of hunger-satiety state. This result suggests not only a reciprocal inhibitory influence between these structures but also a preferential involvement of these two structures in the two components of reward respectively. Neural activation of different OFC sub-regions was correlated with either liking or wanting ratings, suggesting a role of OFC in coding of the magnitude of reward. Finally, during odor reward processing, activity in the anterior insula showed negative correlations with body-mass Index and activity in the ACC was higher for food odors than for non-food odors. Our results confirm in humans that different neural structures are involved in different aspects of reward responses. They suggest that the separable psychological constructs of food liking and wanting can be functionally segregated within the cortico-striatopallidal circuit.

Development of a simple taste test using whole mouth tasting method

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Introduction: Taste dysfunction is one of the biggest contributing factors for nutritional intake and for quality of life declining in the elderly or patients with medication. The purpose of this study is to develop a new simple procedure for the evaluation of taste sensitivity evaluation using generally healthy adults as subjects.

Methods: The study was conducted with 63 adults (35 males and 28 females, age range 20's – 60's). Three-ml supra-threshold test solutions (five concentrations for sucrose (sweet), sodium chloride (salty) and monosodium L-glutamate (umami), respectively) were presented in ascending order. Subject exposed a test solution and swirled it for 3 seconds in the mouth, followed by spitting it out. He/she was forced to choose the perceived taste quality from "sweet", "salty", "sour" "bitter" and "umami". Subsequently, he/she was forced to choose the certainty of the perception from "vaguely", "probably" and "clearly". Score for the answer of each test solution was given according to correctness of the perceived quality of taste and certainty of the perception. Zero was given for no sense of taste or incorrect identification of taste quality. For correct identification, points of 1, 2 and 3 were given according to the certainty of "vaguely", "probably" and "clearly". Total score for each quality of taste was regarded as a sensitivity index for each quality of taste. Order of presentation of taste quality was in random but not umami to be first.

Results: All subjects understood the procedure easily, and no serious operational difficulty was observed. The scores for each test solution showed gradual increase with increasing in concentration within each taste quality. Order of presentation did not affect sensitivity indices. Distribution of umami sensitivity index was normal distribution; however, sensitivity indices for sweetness and saltiness did not show normal distribution.

Discussion: A series of test solutions at above supra-threshold concentration and the degree for certainty of the perception of taste quality were used as parameters in this test. Combination of these conditions could prevent false positive/negative results occurring in recognition threshold test without confirmation trials. Furthermore, use of degree for certainty instead of taste intensity scale could also prevent large variance caused by arbitrary use of scale. With this study, it was demonstrated the procedure used was simple and applicable. It will be further improved to be applied to elderly and patients with taste dysfunction.

Peripubertal exposure to male odors influences female puberty and adult expression of male-directed odor preference in mice

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Testosterone-dependent olfactory signals emitted by male are well known to accelerate female puberty in mice (Vandenbergh effect). However, it remains unclear whether these chemosignals also influence adult expression of male-directed odor preference. Therefore, we exposed female mice to intact or castrated male bedding (vs clean bedding as control) during the peripubertal period (postnatal day (PD) 21–38) and measured maledirected odor preference in adulthood. At PD45 or PD60, females exposed to intact male odors, and thus showing puberty acceleration, preferred to investigate odors from intact males over females or castrated males. Females exposed to castrated male odors did not show puberty acceleration but preferred male (intact or castrated) over female odors. Finally, control females did not show any odor preference when tested at PD45, although a preference for male odors emerged later (PD60). In a second experiment, females that were exposed to intact male odors after pubertal transition (PD36–53) also preferred intact male over castrated male odors. In conclusion, our results indicate that peripubertal exposure to male odors induced early expression of male-directed odor preference regardless of puberty-accelerating effect and that induction of male-directed odor preference is not specific to the peripubertal period.FInally, we tested whether individual chemosignals known to accelerate puberty onset can also trigger a preference for male mice.

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

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Members of the OR37 subfamily differ from other vertebrate odorant receptors due to a variety of special features. They are exclusively found in mammals, are highly conserved during evolution and exhibit a unique structural element, indicating that OR37 receptors are tuned to special ligands. In the search for compounds that can activate receptors of the OR37 subfamily it was found that bodily secretions from conspecifics elicited an activation of glomeruli of the OR37 subtypes A, B and C. Monitoring simultaneously the activity of the paraventricular nucleus of the hypothalamus (PVN), a target region of projection neurons from OR37 glomeruli, revealed a significantly reduced level of activity in comparison to controls. The large number of c-Fos positive cells in the PVN of mice that were kept in a clean test box (controls) turned out to be corticotropin-releasing hormone (CRH) cells, which points to an activation of the hypothalamic-pituitary-adrenal axis and a stress response due to the novel environment. The much lower number of activated cells of mice in a box containing bodily secretions from conspecifics indicates a reduced stress response, a phenomenon related to a social buffering effect. Since bodily secretion from conspecifics activated the OR37 system and simultaneously reduced stress-induced activation of the PVN, it was hypothesized that long chain aliphatic aldehydes, the ligands for OR37 receptors, may be able to induce this effect. Indeed, a similar reduced activity in the PVN was found in mice kept in a clean test box and exposed to a mixture of penta-, hexa- and heptadecanal delivered via air stream. These data indicate that the OR37 system may play a role in mediating social buffering phenomena.

Olfactory cues associated with approach and avoidance of foods: a pilot study

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We conducted a pilot online survey to screen for aspects of olfaction that potentially contribute to approach or avoidance of foods in adults. The invitation to complete the survey was sent to two mailing lists of the personnel of the University of Turku (Finland). The survey was completed by 83 individuals (68 women and 15 men) aged 19 to 66 years (mean 33 years). The respondents were mainly nonsmokers (94%), highly educated (72%), urban residents (68%), and omnivorous not restricting consumption of animal products (68%). They were requested to rate their sense of smell (acuity), level of annoyance experienced from everyday odors in general, and familiarity with 18 herbs/spices, to report food aversions and respective sensory perception triggering the aversion, and to complete validated measures Affective Impact of Odor (AIO by Wrzesniewski et al., 1999; score 0 - 3.0) and Food Neophobia Scale (FNS by Pliner and Hobden, 1992; score 10 - 70). Self-rated odor annoyance correlated with FNS score (Spearman's rho = 0.27, $p = 0.01$). AIO score correlated with average familiarity with herbs and spices (rho = 0.22, $p = 0.05$). As expected, self-rated olfactory acuity correlated with the odor annoyance (rho = 0.35, $p < 0.01$) and FNS score correlated negatively with familiarity with herbs and spices (rho = -0.38, $p < 0.001$). The respondents who reported aversion to at least one food ($n = 59$) had marginally higher FNS score than the respondents without food aversions ($n = 24$; 26 vs. 21, $t(81) = -1.74$, $p = 0.09$). Most respondents who had aversion(s) to food(s) (68%) regarded the odor as a cause of the aversion at least in one case. Our preliminary results suggest that olfaction is important in approach-avoidance behavior related to eating. The research continues beyond the pilot during 2014 and results from a larger data will be presented in the meeting. This study was supported by the Academy of Finland (grant #267698 to A.K. and # 252005 to M.S.).

Unique Organisation of Chemosensory System in Salmon Louse (L.Salmonis) Indicates Adaptation To Strictly Parasitic Life Style

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Obligatory ecto-parasite salmon louse (*Lepeophtheirus salmonis*) is the most important parasite problem to date for the salmon farming industry. The ability of salmon louse to find its specific host, mainly Atlantic salmon (*Salmo salar*), is controlled by both physical and chemical cues. While physical cues are important during the localization of host, the chemoreception plays a defining role in host recognition. Through proper identification of host, salmon louse assure itself the nourishment, reproduction and survival in the highly restricted ecological niche.

In the attempt to clarify the genetic aspects of parasitic behavior, we searched the salmon louse genome for genes involved in the chemoreception. Our investigation revealed remarkable organization of these genes, what possibly is the outcome of adaptation to strict parasite life style. Unlike other Arthropods, salmon louse lack seven-transmembrane domain chemoreceptors: Odorant Receptors (ORs) and Gustatory Receptors (GRs), which are consider the main players in chemosensation. In the contrary, 27 putative Ionotropic Receptors (IR) were found, which are the members of protein family implicated in chemical signal detection (Benton et al., 2009). Only five salmon louse IRs, having co-regulatory function, are the orthologs of conserved antennal IRs in other Arthropods. Remaining, divergent IRs appear to be salmon louse specific and may be involved in species-specific activities. Expression of IRs appear/increase greatly in copepodid, which is free-living and infectious stage of salmon louse, actively searching for the proper host. Down-regulation of species-specific IRs by RNAi treatment against co-receptors, reduce the ability of host recognition and settlement.

Our investigation indicate the IRs are the main/only group of receptors involved in signal detection in salmon louse, and this surprising result points towards a special sensory system in the salmon louse.

Co-expression patterns of odorant binding proteins and receptors on the antenna of the malaria mosquito Anopheles gambiae

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In order to find a blood host and to select appropriate oviposition sites female *Anopheles gambiae* mosquitoes rely on olfactory cues which are sensed by olfactory sensory neurons (OSNs) located within morphologically different sensilla. After entry of odorants through cuticle pores of sensory hairs odour molecules are supposed to be captured by odorant binding proteins (OBPs) which transfer them to olfactory receptors (ORs) in the dendritic membrane of OSNs. Genes for about 60 putative OBPs and 79 candidate ORs have been identified in the genome of *A. gambiae* , suggesting that certain OBP-OR combinations are co-localized in antennal sensilla and interplay in odorant detection. We have assessed the sensilla localisation for a subset of OBPs and ORs, which were selected based on a predominant expression in female antenna. Toward this goal we visualized the relative position of OBP-expressing support cells and OR-expressing OSNs by means of two-color whole mount fluorescence *in situ* hybridization (WM-FISH) with combinations of specific riboprobes for two OBPs, two ORs or OBP-OR pairs. The data demonstrate a complex expression-mosaic of OBPs indicating different sensilla types with partially overlapping OBP equipment. For certain OBP-OR and OR-OR pairings we detected co-existence in the same sensillum. For a number of OR-OBP combinations this finding was validated by combining WM-FISH employing an OR-specific antisense RNA probe with whole mount fluorescent immunohistochemistry (WM-FIHC) using an OBP-specific antiserum. Moreover, WM-FISH/FIHC experiments allowed to clearly assign expression of distinct OBP-OR pairs to a distinct sensillum type.

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Endothelin uncouples GAP junction in sustentacular cells and olfactory ensheathing cells of the olfactory mucosa.

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Several factors modulate the first step of odour detection in the rat olfactory mucosa (OM). Among others, vasoactive peptides such as endothelin might play multifaceted roles in the different OM cells. Like their counterparts in the central nervous system, the olfactory sensory neurons (OSNs) are encompassed by different glial like non-neuronal OM cells (nNCs): sustentacular cells (SCs) surround their cell bodies, while olfactory ensheathing cells (OECs) wrap their axons. While SCs maintain both structural and ionic integrity of the olfactory mucosa, OECs assure protection, local blood flow control and guiding of OSN axons toward the olfactory bulb (OB). We previously showed that these non-neuronal OM cells are particularly responsive to endothelin *in vitro*. Here, we confirmed that the endothelin system is strongly expressed in the OM using *in situ* hybridization. Then, we further explored the effects of endothelin on SCs and OECs using electrophysiological recordings and calcium imaging approaches on both *in vitro* and *ex vivo* OM preparations. Endothelin induced both robust calcium signals and GAP junction uncoupling in both types of cells. This latter effect was mimicked by carbenoxolone, a known GAP junction uncoupling agent. However, while endothelin is known for its anti-apoptotic effect in the OM, the uncoupling of GAP junction by carbenoxolone was not sufficient to limit cellular death induced by serum deprivation in OM primary culture. The functional consequence of the ET-1-induced reduction of the GAP junctional communication between OM non-neuronal cells remains thus to be elucidated.

Hormones modulate the olfactory responsiveness

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The sense of smell significantly affects the amount of food consumption and the frequency of food intake. Most animals, such as rodents, rely on the olfactory systems in their daily foraging. The necessity to find suitable food sources is particular urgent when the internal energy reservoir, the body fat content, is running low. The level of body fat is reflected in the concentration of two adipokine hormones, adiponectin and leptin, which are considered as long-term modulators of energy balance. Whereas the leptin concentration correlate positively with body fat content, the level of adiponectin in the circulation is increasing as the fat reserves are declining. The finding that olfactory neurons have a receptor for adiponectin has led to the hypothesis, that adiponectin may alter their responsiveness. In fact, we have found that exogenously applied adiponectin renders the olfactory sensory neurons (OSNs) more responsive to odorants. This increased activity was also reflected on the next level of olfactory information processing, in the olfactory bulb; after hormone pre-treatment significantly stronger neuronal responses to odor stimuli were registered at receptor-specific glomeruli, indicating that in fact the stronger response at the periphery was conveyed to the brain. An application of leptin, in contrast, did not lead to any change in the responsiveness of olfactory neurons to odorants.

Food finding and food consumption is also crucial in an acute hunger situation. The gastric hormone ghrelin is known as an indicator for an acute hunger and supposed to elicit food intake behavior. In the approaches to assess whether ghrelin may also affect olfactory neurons we found that they do express the receptor for ghrelin, GHSR1a. Moreover, monitoring the olfactory responsiveness revealed that after a pre-treatment with nasally applied ghrelin, a standardized olfactory stimulus activated a significantly higher number of sensory neurons and also led to an increased activity in the related glomeruli. The short-term satiety hormone PYY, in contrast, did not show any effect on the olfactory responsiveness. Together the results demonstrates, that hormones which represent conditions of scarce energy reserves or conditions of an acute hunger both render the olfactory system more responsive to odor stimuli and may thereby contribute to cover the energy demand by an improved discovery of food sources.

Vasopressin depresses excitatory synaptic transmission in mitral cells of the olfactory bulb

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Basic mammalian social interactions strongly rely on olfaction in order to recognize individual con-specifics in rodents, sheep, and even humans. The central arginine-vasopressin (AVP) system enhances social recognition in mammals. Thus, disruption of AVP-mediated neuromodulation in the olfactory bulb was found to impair social recognition, whereas central infusion of synthetic AVP enhanced it. AVP is released into the olfactory bulb from local interneurons, and decreases the firing rate of mitral cells in vivo. This inhibiting action has been proposed to filter social odor information to facilitate odor-based social recognition (Tobin et al. 2010, Nature). Here we aim to elucidate the precise mechanisms of AVP release and action in the olfactory bulb. As a starting point, we investigated the effects of AVP on excitatory postsynaptic potentials (EPSP) in mitral cells generated by olfactory sensory neuron input. EPSPs were elicited in horizontal slice preparations of juvenile rats via extracellular stimulation of the olfactory nerve (20-300 µA, 100 µs, 30s intervals). The average amplitude of these EPSPs was 8.3 ± 0.8 mV (n = 9), and they showed a long-lasting plateau phase (half duration > 1500 ms). Bath application of 2 µM AVP for 10 min produced a decrease in the evoked EPSP in 6 out of 9 of mitral cells tested. The average decrease in EPSP amplitude was to 86 ± 4 % (n = 6, $P < 0.05$) of baseline. The action of AVP was fully reversed 5 min after washing out of the peptide (n = 6). We currently investigate dose dependency and receptor specificity of the AVP effect.
Thus, AVP reduces mitral cell excitability most likely via a synaptic mechanism, similar to previous findings in the supraoptic nucleus of the hypothalamus (Kombian et al. 2000, Journal of Neuroendocrinology).

Expression of c-Fos in the anterior piriform cortex after acquisition of conditioned flavor preference

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Taste aversion learning (TAL) and conditioned flavor preference (CFP) facilitate animal survival and play a major role in food selection, but the neurobiological mechanisms involved are not completely known. The present study propose to examine the neuroanatomical bases of CFP using recording neuronal activity by immunohistochemistry for Fos. Three groups (group 1, 2, and 3) of male rats were trained over 8 alternating one-bottle sessions to acquire a CFP *flavor-flavor* induced by pairing a flavor (grape/cherry) with saccharin. In an additional control group (group 4), the same flavors were presented in water without saccharin. The day immediately following training, the animals of groups 1, 2 and 4 were offered the grape flavor (7 ml) in a 15 min session. Two hours after drinking session, the rats were deeply anesthetized and brains were imunohistochemically processed for c-Fos. Two choice-test were given to group 3 and behavioural data showed that the animals in this group were capable of learning the flavor-saccharin association under these experimental conditions. Neurons showing Fos-like immunoreactivity (FLI) were counted in infralimbic cortex (IL), accumbens nucleus, *core* (AcbC) and anterior piriform cortex (aPC). Immunohistological analysis showed a significant higher number of activated cells only in piriform cortex in groups 1 and 2 *vs* group 4. Results obtained appear to indicate that the learning process might have produced a plastic change in the aPC. Further studies are required to determine the functional relevance of piriform cortex in the flavour-taste learning.

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Effect of oral supplementation of L-Arginine at increasing concentrations on PROP bitter taste responsiveness

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The genetic ability to taste bitter thiourea compounds, such as 6-n-propylthiouracil (PROP), varies greatly among individuals and is directly associated with salivary levels of two specific peptides (Ps1 and II-2) belonging to the basic proline-rich protein family (bPRP). In addition, it is known that oral supplementation of the same peptides, as well as that of the free amino acids (L-Arg and L-Lys) that selectively interact with the PROP molecule, facilitates PROP perception, and the effect is most potent in non-taster subjects. Aim of this work was to investigate the possible relationship between PROP bitter taste responsiveness and salivary levels of free L-Arg, and analyse the effect of L-Arg supplementation at increasing concentration on the intensity and latency of PROP bitter taste responsiveness.

Forty-nine subjects (12 males, 37 females, age 28.6 ± 0.86 y) were genotyped for *TAS2R38* and classified for their PROP taster status by rating taste perception intensity evoked by PROP and NaCl solutions. Salivary levels of L-Arg were determined by HPLC-ESI-MS analysis. PROP bitterness intensity and latency were assessed before and after oral supplementation of L-Arg at increasing concentrations.

ANOVA showed that the levels of L-Arg were significantly higher in saliva of super-taster subjects than in medium tasters and non-tasters. Supplementation of L-Arg increased PROP bitterness intensity and decreased its bitterness latency as a function of the concentration tested. The effect on bitterness intensity was limited to medium tasters and non-tasters, while that on latency to non-taster subjects. These effects were not related to *TAS2R38*.

Our results show for the first time that responsiveness to PROP is strongly associated with free L-Arg, which have a facilitating action on both the PROP perception intensity and promptness with which bitter sensation is evoked. These finding suggest that this amino acid could act by carrying the stimulus to receptor sites, as a transporter of hydrophobic molecules.

Associations between oleic acid flavour threshold, the common SNPs (rs1761667 and rs1527483) in the CD36 and PROP taster status in humans

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Although the association between fatty acid sensitivity and PROP taster status is controversial, several works reported that PROP non-tasters are less responsive to fats, and also have a lower ability to distinguish them in foods. In addition, it is known that fat detection and preference are associated with common variants in the CD36 gene. We analyzed the relationship between two common variants in the CD36 and oral oleic acid flavour threshold in PROP super-taster, medium taster and non-taster subjects. Sixty-four subjects (23 males, 41 females, age 27.6 ± 0.85 y) were genotyped for the two SNPs (rs1761667 and *rs1527483*) in the CD36 by PCR techniques. Subjects were classified for their PROP taster status by rating taste perception intensity evoked by PROP and NaCl solutions. The oleic acid flavour threshold were assessed by a modification of the 3-alternative forced-choice procedure where stimuli were presented in paper disks. In thirty-six subjects, oleic acid orosensory detection threshold was compared with that to the oleic acid esterified to glicerol (triolein).

Subjects homozygous for the G-allele of the *rs1761667* polymorphism had a significant lower threshold for oleic acid than homozygous AA subjects. PROP non-taster subjects had a significant higher oleic acid threshold than super-tasters, although no differences were found among the three PROP taster groups based on the genotype and allele frequencies of this polymorphism of CD36 gene. In addition, PROP non-tasters homozygous for the G-allele had a significant lower oleic acid threshold than homozygous AA subjects, while no differences, related to the *rs1761667* polymorphism, were found in medium tasters and super-tasters. No changes associated with the *rs1527483* polymorphism were found. Threshold values determined for the flavour of oleic acid were lower than for triolein, and both lower than those reported in literature as taste threshold values. These results show that two commons SNPs (rs1761667 and rs1527483) in the CD36 play a role in fat flavour perception, mostly in PROP non-taster subjects. Since it is known that PROP non-tasters have a lower density of taste buds, it will be interesting to study the relationship between number of papillae and genotype of CD36.

Smell But Not Taste Reactivity Is Related To Food Food Neophobia in Toddlers: Results From The Opaline Cohort

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Context and objective: Research has previously identified relationships between chemosensory reactivity and food neophobia in toddlers. However, most studies have addressed this question using declarative data, and without analyzing separately smell and taste. The objective of the present study was twofold. The first objective was to assess the relationships between olfactory reactivity and taste reactivity in toddlers, using experimental designs with different tastants and odorants. The second objective was to determine the relationships between olfactory /taste reactivity and food neophobia in toddlers. The hypothesis was that the higher the chemosensory reactivity of the child, the more the child would be neophobic.

Method: One hundred and twenty-three mother-child dyads from the Opaline birth cohort (Observatory of Food Preferences in Infants and Children) were involved in the study. A validated parent-administered questionnaire (Rigal et al., 2012) was used to assess children's food neophobia. The children's taste reactivity was based on the variance of intake scores obtained for the five basic tastes (Schwartz et al., 2009). The children's olfactory reactivity was based on the variance of mouthing scores obtained for 4 bottles bearing pleasant food odours and 4 bottles bearing unpleasant food odours (Wagner et al., 2013). All these measures were collected when children were between 20 and 22 months of age. Kendall correlations were calculated to evaluate the relationships between these variables.

Results: The Kendall correlation between olfactory reactivity and taste reactivity in children was not significant. Neophobia scores were modestly but significantly associated with olfactory reactivity, but not with taste reactivity.

Discussion: The present study highlights the need to study the links between eating behaviours and olfactory/taste reactivity separately. It also suggests that more neophobic children, in comparison to less neophobic children, are more responsive to odours, but not to tastes. Thus, the rejection of unknown foods before ingestion, which is common in neophobic children, could be partly due to their odours.

Major Urinary Proteins As Metabolically Active Scaffolds Within The Mouse Body

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Chemical communication in the mouse involves the excretion of signalling molecules in various body fluids. Different families of chemosignals are carried outside the male mouse body with urine. Some of them have already been characterized, including various volatile odorant molecules, different peptides and the Major Urinary Proteins (MUPs). Urinary MUPs are synthesized in the liver and excreted in the urine, after crossing the glomerular barrier and escaping proteolysis in the proximal convoluted tubule of the kidney. Production and emission of male chemosignals require functional, behavioral and anatomical specialization in the emitter, which are believed to depend on testosterone. On the other hand, MUPs may influence mouse metabolism. We asked whether the mere excretion of MUPs could influence the male mouse physiology and behaviour. To this end, twice daily we injected MUPs either purified from adult male urine or expressed in an heterologous system in castrated mice. After ten days, we monitored their micturition behaviour, luteinizing hormone and testoserone and evaluated the morphology of kidneys. Similar results were obtained with purified or recombinant MUPs. MUPs injection induced a sudden increase in food and water consumption, supporting a role for MUPs in modulation of metabolism. Kidney weight also increased in MUPs-injected castrated mice, showing that MUP excretion could selectively impact on the kidney function, while not affecting overall body weight or other organs. While MUPs injection did not impact on testosterone levels, that remained low in castrated whether they received MUPs or not, the circulating LH increased after MUPs injection. Lastly, while castrated mice were slower than intact male mice in releasing urine when put in a novel environment, MUPs-injected castrated mice were as fast as intact mice in releasing urine. Therefore, the injection of MUPs was sufficient to alter micturition behaviour, independently of testosterone.

A CD36 expressing subset of murine olfactory neurons is involved in milk detection.

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Olfactory signals influence food intake in a variety of species. In the first days of life mammals feed on breast milk, and survival in this period critically relies on olfactory detection of milk component. However, the molecular identity of receptors and ligands mediating olfactory-dependent milk recognition remain poorly understood. We started to elucidate the molecular requirement for milk recognition in suckling mice.
Here, we report that olfactory neurons expressing the fatty acid receptor CD36 are required for the detection of oleic acid, a major milk component. The subpopulation of olfactory neurons that are characterized be expression of CD36 share olfactory-specific transduction elements and project to numerous glomeruli in the ventral olfactory bulb. Ca2+ imaging showed that olfactory responses to oleic acid are drastically reduced in mice lacking CD36, which also showed a reduced weight gain during the suckling period. In addition, using STED microscopy we demonstrate a receptor-like localization of CD36 in olfactory cilia. Strikingly, careful inspection of the olfactory system at different ages revealed that CD36 is present in olfactory cilia only during the time the animals feed on milk. Regulation of the intracellular localization of CD36, which facilitates the olfactory detection of lipid-like olfactory ligands, seems to play a crucial role in adaptation of mammals for their nutritional needs during the first weeks of life and constitutes a remarkable example of the main olfactory system plasticity in the first days of life.

Engagement in Olfaction-Related Activities is Associated with the Ability of Odor Identification and Odor Awareness

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Recent research has shown that within-gender variability in olfactory abilities may be linked to sexual orientation, particularly in men, but is better predicted by childhood gender nonconformity. However, whether there could be similar within-gender variability in odor awareness remains unclear. Further, gender differences in olfactory abilities and odor awareness in favor of women have been proposed to be partly related to women's broader olfactory experience due to their greater engagement in olfaction-related activities. Nevertheless, within-gender variability in odor exposure could also be expected. Therefore, in a sample of 156 men and women (83 non-heterosexual), we aimed to look for between- and within- gender variability in odor awareness and self-reported engagement in specific olfaction-related activities. Secondly, we tested whether interindividual (between- and within-gender) differences in olfactory abilities and odor awareness might be related to experience with odors, assessed in terms of engagement in olfaction-related activities. The results of the present study show that within-gender variability, previously found in some olfactory abilities in men and women, does not seem to extend to odor awareness, and appears to only apply to certain olfaction-related activities. In the total sample, more frequent exposure to a greater variety of potentially intense or novel food odors and flavors in both childhood and adulthood was positively linked to both greater odor awareness and better odor identification. There was also a positive link between female stereotyped activities in childhood and odor awareness. Our results suggest that long-term everyday experience with odors may be linked to a better ability of odor identification and greater odor awareness, although longitudinal studies are needed to further investigate these associations.

Combinatorial and genotype specific olfactory code is based on relative ratios of the major urinary protein (MUP) isoforms and appears at juvenile stage of the mouse development

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Major urinary proteins (MUPs) of the house mouse form a large group of highly polymorphic acidic isoforms with molecular masses of 18-20 kDa. MUPs are encoded by the *Mup* gene cluster, which consists of about 35 genes and pseudogenes and is mapped to chromosome 4. Nowadays MUPs are considered as a key component of the mouse olfactory signature which can provide all essential information about individuality of donors (e.g. Churakov et al., 2013; Kaur et al., 2014; Novikov, 2014). We examined ontogenetic profiles of MUPs expression in male and female mice of CBA/LacJ and C57BL/6JY strains using electrophoresis in polyacrylamide gel (PAGE). Quantitative evaluation of eight MUP isoforms (*A-H*) revealed that each genotype is characterized by specific *combinations* and different *relative ratios* of the same MUP fractions and forms two distinct subsets (modules) according to stability of their ratios during mice ontogenesis. The first subset, which could be characterized as «ontogenetically stable», consists of fractions *A,C,D,* and *E;* the second subset consists of fraction *B,F,G,* and *H,* which are significantly influenced by age factor. This probably reflects their different functional roles in creation of genotype- and sex-specific olfactory images. The «*adult proportion*» profile of different MUPs appears in animals of two lines very soon after weaning. Our data suggest that the pattern of *Mup* gene expression during mouse ontogenesis is regulated through a very stable genetic program. These results also suggest that genes encoding MUP production are functionally active at juvenile stage of the mouse development. The differential and combinatorial pattern of *Mup* genes expression during ontogenesis resembles a 1D «bar code» and seemingly works like a genotype- and gender-specific module. We propose that various pheromonally active volatile organic compounds (VOCs) represent «letters» of the chemical «language» in *Mus musculus* L. and that, correspondingly, specific *combinations* (modules) of MUPs efficiently organize these «letters» into readable «words» that encode sex, age, physiological state, hierarchical status, and genotype. We suggest that these specific modules of MUPs are subsequently screened and recognized by *complementary* vomeronasal V2Rs neurons of the recipients, and these processes constitute the basis of pheromonal coding and decoding in *Mus musculus* L. by MUPs↔V2Rs interplay.

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Dynamic coding of different tastes in the human brain

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In spite of decades of research, we know very little about the cortical dynamics during gustatory perception in humans. Only a marginal number of event-related potentials (ERP) studies has been conducted on taste so far owing to difficulties in stimulus control; the few results suggest differences in processing time for different tastes. Independently, differences in behavioral response times have been reported for different tastes. Whether the latency differences at the behavioral and neuronal levels are related remains unclear. We measured multi-channel head-surface electroencephalographic (EEG) in human participants while they received liquid tastants inducing salty, sweet, sour, or bitter sensations. Participants were passively tasting in one study and responding as quickly as possible to the tastes in another study. Electrical neuroimaging analyses were performed to characterize the time course and spatial distribution of the electric field at the head-surface level and physical-mathematical inverse solution and head models served to estimate the underlying active neural sources. The ERP yielded latency differences between tastes that varied depending on whether participants tasted passively or performed a speeded taste detection task. Notably, responses to salty and sour were clearly faster than responses to sweet and bitter. In the speeded task, neuronal response latencies correlated with behavioral responses latencies. When accounting for the difference in response latencies, source analyses revealed similar, yet not identical, cortical activations of the insula and opercula, superior temporal gyrus, ventromedial orbitofrontal and cingulate cortex for all tastes. Together, the results suggest that a common cortical network is activated by different tastes in a dynamic manner and that these dynamics, furthermore, interacted with participants' behavioral goals.

The scent of disease

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Ability to detect diseases in conspecifics would be advantageous for the individual. In line with this, rodents avoid body odors of infected individuals. Two studies (Olsson et al. 20014; in prep.) indicated that this is possible by way of human smell and human observers. T-shirts from donors (worn for 4 hours) that had received an injection of endotoxin [0.8 ng lipopolysaccharide (LPS) / kg body weight], which causes systemic inflammation, smelled more unpleasant, intense, and sick than shirts from donors that had received a placebo (Saline) injection. GC/MS analysis of the shirts suggested that the change of body odor was not due to a general increase of odorous compounds in the "sick shirts" compared to "placebo shirts" but rather to a qualitative change. Study 2 (ongoing) further investigated the nature of this perception. In a first experiment, we compared the body odor of 30 endotoxin (0.6 ng LPS / kg body weight) and 21 placebo (Saline) donors. Again, body odors were sampled during 4 hours using T-shirts. Observers then smelled the shirts and rated intensity, pleasantness, and disgust. In a second experiment, urine from these donors were collected and was investigated in the same way with subjective ratings. Altogether the data suggest that systemic inflammation makes body odors more aversive within a few hours.

Decreased taste perception in young population and their relationship with obesity

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Introduction. Obesity is a public health problem that affects the quality of life in the worldwide population.

Objectives. The study aims to correlate the loss of sweet taste sensitivity with the obesity and overweight, with biochemistry markers and anthropometric markers.

Materials and Methods. Involved 200 individuals, 18 to 25 years old. The Palatability test was performed for each individual, focused on sweet or umami taste molecules like phenylalanine, sucralose, sucrose and fructose syrups. Were measured height, weight, waist circumference, hip circumference, determining body mass index and waist to hip ratio. Glucose, glycosylated hemoglobin, urea, creatinine, uric acid, cholesterol and triglycerides were determined.

Results . 80 peoples who have anthropometric overweight or some degree of obesity, have a significantly different pattern for the taste to all the sweet taste molecules, compared to 120 peoples with normal body mass index and their perception towards sweet tastes, well as a significant increase in the levels of biochemical glycemic index and lipid profile in obese people.

Conclusions. The results indicate the relationship between loss of sweet taste perception and an increase in the values of glycemic index and lipid profile, placing the overweight individual in to a complication in the taste perception and health implications, in which case the control of food intake plays an important role as obesity, diabetes mellitus and dyslipidemia in young population.

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Selectivity of C57BL/6 and BALB/c newborn mice to the odors of milk and nipple differing in lactational age and strain.

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In mammals, odor-based communication between females and her newborns is fundamentally crucial for neonatal survival and adaptive development. Globally, female's body emits a multitude of salient sensory cues, and more especially, some olfactory cues originating from her mammary areas and lacteal fluids, which support infant orientation towards her nipples and adaptive responses. However, covariation between maternal sensory cues potency and infant sensory perception during the development are still poorly investigated.

The present study aimed to address whether there are periods in lactation during which milk would convey higher behaviorally-active olfactory components for newborns mice and whether milk would have a potential role in the control of the selective nipple attachment behavior in an ecological context of suckling. Basically, the principle of this study consisted in exposing 2 day-old pups from two laboratory mice strains, i.e., C57Cl/6 and BALB/c strains, to milk collected from lactating females (LF) on different lactational stages and/or strains, or to intact nipples of LF differing in lactationnal stages and/or strain. Investigating the infant responsiveness from two laboratory mouse strains provide us firsts elements to verify on whether this sensory mechanism is representative of the *Mus musculus* species.

Our findings demonstrated that both C57Cl/6 and BALB/c newborn mice aged of 2 days exhibited a greater attraction to the odors of early-lactation milk compared to late-lactation milk regardless of the strain female donor. Moreover, experiments revealed that newborn mice showed a more efficient oral grasping nipple towards early-lactation LF than late-lactation LF from their own strain. Nevertheless, C57Cl/6 2 day-old pups displayed this selective response when they were also exposed to an alien-strain LF, whereas BALB/c 2 day-old pups expressed an identical nipple oral grasping to early and late-lactation LF from the alien strain.

Such differential response seems to indicate some lactation stage-related and strain-dependant specificities in odor properties of milk and of nipple, and in chemosensory abilities of mouse pups.

Differential binding of the Pheromone binding protein 1 (PBP1) and General Odorant binding protein 2 (GOBP2) of Bombyx mori toward the main components of the pheromone blend in air.

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Pheromone binding protein 1 (PBP1) and General odorant binding protein 2 (GOBP2) are two members of the insect odorant binding protein family. These proteins are abundant in the antennae of the silkmoth *Bombyx mori* , where they are involved in the perception of semiochemical stimuli. PBP1 and GOBP2 are differentially expressed between the males and females of the *B. mori* species. PBP1 is mainly present in the long sensilla trichodea of males, which respond to the sex pheromone components bombykol and bombykal. GOBP2 is found instead in the female antennal structures that are stimulated by compounds present in the environment, such as linalool and benzoic acid.

Fluorescence binding assays show that both PBP1 and GOBP2 bind bombykol as well as bombykal with dissociation constants of the order of 10^{-8} M. However, GOBP2 has a slight preference towards bombykal.

Here we demonstrate that PBP1 and GOBP2 respond to bombykol and bombykal vapours differently, when tested in air. Quartz crystal microbalances (QCMs), with a resonance frequency of 20 MHz, were used as platforms to evaluate the response of the two proteins to the pheromone components in vapour phase. QCMs are highly sensitive mass transducers able to detect small variations in the mass occurring on their surface.

PBP1 and GOBP2 were grafted on both gold electrodes of QCMs using Self-assembled monolayers of thioctic acid (TA) and tested against a range of vapour concentrations of bombykol and bombykal.

The PBP1-based biosensor showed a high affinity toward both pheromone components, while the GOBP2-based biosensor had a much less substantial response. The resulting biosensors were highly sensitive to the compounds, considering the low vapour pressure of bombykol and bombykal.

The results indicate that PBP1 and GOBP2 may have very different roles in the perception of the olfactory stimuli, which is also supported by the different expression patterns found for these two proteins in *B. mori* .

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The influence of maternal body odors on preterm infant development

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Preterm infants (PIs) have to undergo rapid neurodevelopment to qualify for discharge from hospitalization. Immediately after birth, the PI is hospitalized in a neonatal intensive care unit (NICU). This environment is strikingly different from womb ecology: light, sound and visual stimulation are abundant, and the PIs experience a great deal of stressful events mainly in the form of medical interventions. The consequences of such constant stress are associated with decreased neurodevelopment as well as down-regulation of homeostatic mechanisms via sympathetic nervous system activation.

As early as the 28th week of gestation, the olfactory system of the fetus is mature enough to allow chemoreception. Exposure to different odors from this age and on alters PI behavior, reducing stress, pain and apnea. Several odors, including body odors (BO), are known as effectors of the parasympathetic nervous system (PNS). Shortly after birth, a newborn is able of recognizing its mother's BO, but the natural exposure of PI to its mother's BO is reduced significantly while in NICU. We hypothesize that long-term exposure of a PI to its mother's BO can reduce stress levels through PNS activation and thus improve physiological status, eventually resulting in a shorter period of hospitalization (POH).

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

Signal transduction and amplification in the confined cilia of olfactory neurons

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The sensory units of an olfactory receptor neuron (ORN) are long and slender cilia, where odorant recognition and transduction takes place. Contrary to phototransduction, the detection of odorant molecules does not depend foremost on a biochemical protein amplification cascade. Instead, odorant exposure leads first to the opening of Ca^{2+}-permeable cyclic nucleotide-gated (CNG) channels. The subsequent Ca^{2+} influx leads to the opening of Ca^{2+} activated Ano2 chloride channels that generates a large chloride current which is the major amplification step. In the small ciliary volume of around 1 femtoliter the exchange of a single ion already leads to a concentration change around 2nM. Thus, currents can rapidly shift reversal potentials and change the electrical driving forces. How does this affect the olfactory response? Why is the current carried first by cations and then anions? What is the role of the chloride current?

To study such questions we developed an electrodiffusion model that accounts for the biochemical transduction pathway and the complex ion dynamics in the confined cilium geometry. This model allows studying the olfactory response in great detail by dissecting the contributions of the various ionic fluxes and the biochemical reactions. Simulations reveal complex ion dynamics in the cilia. For example, the current fraction carried by various ions is not the same for the currents between cilia and mucus compared to cilia and cell body. Furthermore, the simulations suggest that the characteristic initial peak of the odorant response is not generated by the transduction pathway, but is an electrical property due to the ion dynamics.

Major Urinary Proteins tailored to detect selected compounds as tools for developing biosensors

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The creation of novel mutant proteins targeted to bind different chemical ligands is possible by computational manipulation. In terms of protein binding functionality, the recognition between two molecules can be predicted even with atomic scale accuracy using this approach.
We analyzed the aminoacidic sequence of the Major Urinary Proteins (MUPs), 18 kDa proteins excreted in mice urine and able to bind odorants, exploring their ligand binding affinity via *in silico* methods.
The protein mutation was performed on MUP20 (AKA Darcin), an isoform routinely expressed in our lab. We focused the mutations on those residues lining the binding pocket, that highly interact with a group of selected volatile compounds.
We defined each mutant by an unique change on a single aminoacidic residue of MUP20, obtaining a final selection of 11 novel mutants. Next, the original crystal form of MUP20 (PBD ID: 2L9CM) was modified in accordance to each mutation.
To predict the binding profile between wild type/mutants MUP20 and the selected ligands, we used the docking method (EADockDSS software), through interpolation of the chemical data of each crystal model across every possible interaction with the set of volatile compounds.
The binding modes that allow the ligand to reach the pocket were analyzed by calculating the strength of the interaction represented by the energy required to afford the binding.
Subsequently, the mutant proteins were expressed in BL21 strain of *E. coli* through a plasmidic vector encoding a synthetic gene, holding the *in silico* designed sequence.
A fluorescence-competitive binding assay, using the same set of volatile compounds as the *in silico* approach, was conducted according to a described protocol (Sun *et al.* , 2012), to test *in vitro* the binding profile of mutant proteins.
The statistical analysis of *in silico* and *in vitro* data was carried out using a clustering method, the Principal Component Analysis, extracting a few top components as a result of clustering the binding behavior of each mutant protein. We obtained three separated components or groups of mutants, in other words, distinctive binding profiles in terms of differences with the wild type MUP20.
These results suggest that minimal modifications in the aminoacidic sequence of the wild type MUP20 can produce a significant change in the binding profile of the protein as our data showed. Hence, this could be an important advantage for developing customized MUP-based biosensors sensitive to a wide range of volatile molecules.

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A1 receptor impact on neuronal processing in the mouse olfactory bulb

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Neuromodulation by adenosine is crucial for the tight regulation of neuronal activity in many brain regions. In the olfactory bulb (OB), the precursor for adenosine, ATP, is released as a co-transmitter by olfactory receptor neurons (Thyssen et al., 2010). Additionally ATP-degrading enzymes are highly expressed in the OB suggesting a function of adenosine in the OB network. To analyse a potential influence of adenosine on OB neurons we performed whole cell patch-clamp recordings on mitral cells using them to monitor overall network activity. Bath application of adenosine reversibly reduced the frequency of spontaneous synaptic inputs in mitral cells, indicating an effect of adenosine on OB neurons. DPCPX, a specific antagonist of A1 receptors (A1R), blocked this effect pointing towards an A1R-mediated mechanism. Using A1R knockout mice we show that adenosine hyperpolarises mitral cells in an A1R dependant manner. A direct action of adenosine on mitral cells is supported by *in situ* hybridisations showing an expression of the A1R only in mitral/tufted cells. Further investigations reveal that the hyperpolarising effect of adenosine on mitral cells is mediated by a potassium-driven outward conductance. Known A1R-modulated potassium channels, including GIRKs, were tested by use of specific blockers. However none of the used blockers reduced the adenosine-evoked hyperpolarisation and the identity of the potassium channel remains unknown. Mitral cells do not only receive synaptic input from most cell types in the OB, but also critically drive the neuronal network and integrate odour information before sending it to higher brain areas. Thus, the modulation of mitral cells by adenosine affects the whole OB network. To analyse the influence of adenosine on the input-output relation of odour information in mitral cells we stimulated the axons of olfactory receptor neurons, thus mimicking incoming odour information, and measured the resulting output behaviour of mitral cells. In the presence of adenosine the signal-to-noise ratio, as measured by the ratio of evoked/basal action potential firing was improved. So far the data suggest that adenosine reduces overall background noise by silencing mitral cell basal activity, thus improving the signal-to-noise ratio for mitral cell output.

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Iso-energetic diets containing β-glucan or arabinoxylan show different expression of Tas1R3 and similar expression of fatty acid sensing genes in the porcine gastrointestinal tract.

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Recent studies have uncovered several fatty acids (GPR40, GPR41, GPR43, GPR120 and GPR84) and carbohydrate (T1R2/T1R3) receptors which respond to short, medium and long chain fatty acids and simple sugars present in the diet, respectively. Fibrous dietary compounds have the ability to increase the viscosity of digesta which slows down the flow of digestive enzymes and the absorption of nutrients. For example, soluble dietary fibre reduces glucose absorption (and the glycaemic index) and other macronutrient absorption such as fatty acids. Thus, we hypothesize that high dietary fibre may limit fatty acid and sugar availability which, in turn, will increase the expression of their sensing receptors. The purpose of this study was to evaluate the effect of dietary addition of soluble fibre compounds arabinoxylan (AX) or β-glucan (BG), on the expression level of fatty acids and sugar nutrient sensor genes in the porcine gastrointestinal tract (GIT).

For two weeks, 18 Large-White male pigs were assigned to three iso-energetic diets, differing on their fibre type and content. The three diets consisted of: a control (CTR) based on wheat starch, and two experimental diets containing 10% wheat AX or oat BG substituted for some wheat starch. The mRNA was extracted from collected tissues and qPCR followed by statistical analysis were carried out according to the Pfaffl method. The taste and fatty acid sensor gene amplicons were assayed for five selected tissues: circumvallate papilla (CV), stomach ridge, jejunum, colon distal and caecum.

The results showed significant expression of fatty acid receptor genes along the porcine GIT particularly outside the oral cavity. Compared to the other fatty acid sensors, GPR41 and GPR43 had different expression patterns across tissues showing a significantly ($P<0.05$) higher expression in caecum or stomach than in CV. In addition, Tas1R3 was also abundantly expressed in and outside the oral cavity. In contrast, the Tas1R2 expression pattern was found to be site-specific, with higher ($P<0.05$) levels in the tongue compared with the other tissues. The presence of high dietary BG significantly ($P<0.05$) increased the expression of the Tas1R3 in stomach when compared to the AX. Overall, our findings show significant expression of fatty acid and sugars receptors along the porcine GIT which was not affected by the fibre level in iso-energetic diets. However, the effect of fibre type on specific receptor genes (Tas1R3) relevant to carbohydrate sensing, warrants further investigation.

The evaluation of olfactory function in individuals with halitosis

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Objective: Halitosis and olfactory dysfunction may disrupt an individual's quality of life remarkably. The aim of this study was to evaluate the olfactory abilities of subjects with halitosis evaluated using the measurements of volatile sulfur compounds (VSCs).

Material and Methods: This study was carried out in 77 subjects, with a mean age of 40.1 ± 13.3 years, ranging from 18 to 65 years of age. Forty three participants were diagnosed as halitosis according to the gas chromatography results and constituted the halitosis group. Also, a control group was created from individuals without a complaint of halitosis and also who had normal values for VSCs. Each subject's orthonasal olfactory and retronasal olfactory functions were assessed using "Sniffin' Sticks" and retronasal olfactory testing.

Results: Odor threshold scores were lower in participants with halitosis as compared to controls. Also, hyposmia was seen more common in the halitosis group than in controls. Moreover, a significant negative correlation was found between odor threshold scores and VSCs levels, particularly with hydrogen sulfid and dimethyl sulfid levels.

Conclusions: The presence of VSCs may have a negative effect on olfactory function.

Body Odor and Perfume of Caregivers are Salient to Typically- and Atypically- Developing Young Children

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Humans react to conspecific body odors, which informative value was often assessed in mate attraction, but less so in parental/filial interactions. Body odors are usually mixed with perfumes (P), both components conveying cues. Thus, early responses to either odor component are a way to investigate the ontogeny of olfactory social cognition. Here, two studies focused on typical children's ability to recognize either maternal body odor or P. A 3rd study probed whether this ability generalizes to other caregivers and to atypically-developing children.

In study 1, 32 children (3-4/5-6 y) were exposed to 4 t-shirts, one previously worn by the mother. Based only on olfaction, they had to: 1/ recognize which was their mother's, and 2/ report the most preferred. Among 3-4 and 5-6 y-olds, 81 and 100%, respectively, recognized the mother's t-shirt, whereas for 81 and 61%, respectively, the most preferred t-shirt was their mother's. Thus, 3-6 y-olds olfactorily recognize their mother, 5-6 y-olds being better recognizers than 3-4 y-olds, but displaying less preference for her t-shirt.

In study 2, 32 children (5-6 y) were exposed to 4 P, including their mom's. First, unaware of their mom's P inclusion, they fulfilled: 1/ preference tasks, 2/ evaluation of their likely use of each P on themselves, and 3/ recognition of mom's P after being told that it was included. Children liked all P, without advantage for their mother's. But 70% of them chose their mom's P as the one they would apply on themselves. Finally, 50% of them recognized their mom's P ($p < 0.05$). Thus, 5-y-olds recognize their mom's P at a better-than-chance level, do not express any clear preference for it, but favor it to self-anoint.

In study 3, 4 blind, multihandicapped children (aged 3-6 y) were trained for 10 d to associate a given P with their educator. After conditioning, they were filmed when exposed for 30 s to 4 P, including the familiar one. Six judges rating the children's attention, sniffing, and smiling responses agreed that 3 of 4 selectively reacted to the P associated with their own educator.

In conclusion, young children sense their mother's odors, showing recognition and explicit or implicit preference. P appears as efficient as natural odor to elicit recognition and emotion in typical and multi-handicapped children, and both natural and artificial odorants may thus be used to manage child-caregiver interactions.

Modulation of Dendrodendritic Synaptic Transmission in Olfactory Bulb Mitral Cells by Adenosine

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Besides their predominant role in energy metabolism as well as DNA/RNA synthesis, purines such as ATP, ADP and adenosine play a major role in the communication between cells in various areas throughout the nervous system. In the olfactory bulb (OB), ATP is released by olfactory receptor axons as a neurotransmitter and stimulates neuronal network activity as well as glial calcium signalling (Lohr et al. 2014). In addition, ATP-degrading enzymes are highly expressed in the OB, suggesting a pivotal role of purinergic modulation in olfactory information processing.

In this study, we analyzed adenosine-mediated modulation of signal transmission between neurons of the OB in acute mouse brain slices. We studied dendrodendritic inhibition (DDI) in mitral cells, the principal neurons projecting to higher brain centers, by using whole-cell patch-clamp recordings. DDI plays a major role in the processing of olfactory information and is thought to be mediated mostly by recurrent synapses between glutamatergic MCs and GABAergic granule cells. DDI was evoked by a brief depolarizing voltage step and was greatly enhanced by magnesium-free extracellular media and application of cyclothiazide to enhance synaptic transmission mediated by NMDAR (mainly expressed by granule cells) and AMPAR (mainly expressed by other types of interneurons), respectively. Bath application of adenosine led to a reduction of NMDAR-mediated DDI by 20%, indicating that adenosine affects the reciprocal synapse between mitral cells and granule cells. We also investigated isolated AMPAR-mediated DDI currents. These currents were blocked by 80% using NASPM, a specific antagonist of calcium-permeable AMPARs, which are particularly expressed in parvalbumin (PV) interneurons. Adenosine reduced NASPM-sensitive DDI currents by 15%, illustrating an impact of adenosine not only on mitral cell-granule cell synapses, but also on mitral cell-PV interneuron synapses. Furthermore, adenosine application resulted in a 35% reduction of mitral cell self-excitation, used as a quantification of glutamate release from mitral cell dendrites. To unravel the mechanism by which adenosine affects DDI, we measured voltage-gated calcium currents in mitral cells. In the presence of adenosine, voltage-gated calcium currents were reduced by 20%. This suggests a presynaptic modulation of reciprocal synaptic transmission by inhibition of calcium influx into mitral cells, thus reducing the release of glutamate from mitral cell lateral dendrites. This, in turn, leads to a decrease in GABA release from granule cells and PV interneurons and, hence, in DDI currents in mitral cells. The results show that adenosine influences the performance of dendrodendritic synapses between mitral cells and different classes of interneurons, which might contribute to modulation of olfactory information processing.

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Individual Olfactory fingerprints

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A key step of linking neuronal olfactory coding to odorants physicochemical properties is to account for individual variability in odor perception. Therefore, one would like to parameterize individuals in a space of odor preferences to account for this variability, i.e. instead of representing odors, representing people and quantify their individual olfactory percept. We collected perceptual ratings (using VASs) from 61 participants who rated 32 odorants along 57 descriptors. Twenty seven of the descriptors were taken from Dravnieks' odor atlas and additional thirty two were selected through a semantic analysis process. Using only 15 odorants and 30 descriptors we were able to characterize an 'Olfactory fingerprint' for an individual. This fingerprint allowed us to identify a given individual out of the pool of 61 individuals with 90% accuracy. The 'Olfactory fingerprint' was generated by normalizing answers per subject per odor and then averaging across odors, i.e. generating a specific descriptor usage profile which is independent of the odors used (averaged across odors). To test if this profile is not merely a reflection of semantic usage of descriptors, we created an odor similarity matrix for each subject that is independent of the descriptors used. The subject's similarity matrix was generated by correlating all odor ratings within a subject. Using a same method on the similarity matrix we showed again that we are able to identify individual participants with 90% accuracy, thus showing that the profile created was not specific to the descriptor usage.

Involvement of CCK in normal gustatory responses to bitter compounds

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Cholecystokinin (CCK) is a gut peptide hormone released from endocrine I cells in response to nutrient ingestion. The major physiological role of CCK is recognized in modulating gastric emptying, stimulating pancreatic enzyme secretion. Moreover CCK is involved in the regulation of food intake, satiation, and energy balance. This effect is mediated through binding to CCK receptors(CCK-AR and CCK-BR) on vagal afferent neurons. While CCK is most abundant neurotransmitter peptides expressed in the brain. Recent studies demonstrated that bitter receptors (T2Rs) are expressed in the gut enteroendocrine cells and CCK is secreted from the enteroendocrine cells by bitter taste stimuli. Also in the taste tissue, CCK is shown to be expressed in a subset of taste cells which co-express gustducin, an alpha G-protein, involved in signal transduction for bitter, sweet and umami tastes. These evidences suggest a possibility that CCK may also play important roles in the taste organ.To address this issue, we first asked expression of CCK, CCKR (A, B) in taste bud cells and the geniculate ganglion of wild-type mice by RT-PCR, in situ hybridization and immunohistochemistry. We next asked involvement of CCK in taste responses by comparing chorda tympani (CT) nerve responses of wild-type, CCKAR-KO, CCKBR-KO and CCKR-WKO mice to various taste stimuli. Then, we tested potential direct action of CCK on the taste nerve endings by examining changes in CT nerve activities to injection with CCK from the femoral vein. Finally, we asked involvement of CCK in behavioral responses to taste stimuli by using a short time lick test for 10 seconds in CCK-ARKO, CCK-BRKO and CCKR-WKO mice. As a result, we found that CCK and CCKR (A, B) are expressed in a subset of taste cells and cell bodies of the geniculate ganglion. Furthermore, we also found that the i.v. injection of CCK increased activities of CT nerve in a dose-dependent manner. CCK receptor KO mice exhibited reduced lick responses to bitter compounds but not to the other taste stimuli. Collectively, these findings suggest that CCK may be involved in normal bitter taste responses in mice.

Habituation to 32 odor molecules varying in chemical, physical and psychophysical characteristics

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Habituation is a form of non-associative memory, serving to reduce responsiveness to continuous or repetitive stimuli. This process is involved in all sensory modalities and is so fundamental that our brain would "burn-out" without it. In general we were interested in understanding what cues the brain uses to specifically reduce olfactory activation in response to prolonged odorous stimulation. More precisely, we studied habituation towards 32 odors differing in chemical, physical and psychophysical characteristics. Fifty eight healthy subjects (age 26±5 years; 33 women) evaluated continuously during 120s the intensity of each odor, presented during a series of 4 sessions. After matching intensities, the odors were delivered to participants using a computer-controlled olfactometer. Intensity was recorded with a device which digitalizes the pressure applied to a syringe into intensity values. Relaxation or compression of the syringe is equal to a decrease or increase of intensity, respectively. As a first result we observed a wide inter-individual variation of the habituation curves and relatively little intra-individual variation. To reduce this variability that risked to mask the effects of habituation, only subjects were analyzed who presented a constant pattern of habituation across odors. Subjects who did not indicate habituation to more than 21 odors were not included in the analyses (n=16). Individual curves were then pre-processed, mean were calculated and 10 variables defining almost perfectly the curves were extracted. A principal component analysis (PCA) was performed to define a "habituation" space based on the 10 variables. Hierarchical clustering (HC) was run on the 10 habituation variables plus 13 additional ones constituted of psychophysical, chemical and physical variables. Two dimensions explained 48% of the variance. The separation of the odours in three clusters presented the best inertia gain. Two of the clusters differed in habituation patterns; one was a group of weak habituation odors while the other was a group of odors that produced a strong habituation. These two clusters presented a higher trigeminality and intensity for the first group and a weaker trigeminality and intensity for the second group. From this first analysis, trigeminality appeared as the first determinant that reduces habituation. In a second analysis the trigeminality effect was virtually removed by weighing of the habituation variables and a second PCA/HC was run. This second habituation space also presented 3 clusters - two groups were, respectively, composed of odors producing strong or weak habituation. These clusters correlated with the factor functional group.

Olfactory Impairment and Subjective Olfactory Complaints Independently Predict Conversion to Dementia

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We examined whether conversion to dementia can be predicted by self-reported olfactory impairment and/or by an inability to identify odors. Common forms of dementia involve an impaired sense of smell, and poor olfactory performance predicts cognitive decline among the elderly. We followed a sample of 1529 participants, who were within a normal range of overall cognitive function at baseline, over a 10-year period during which 159 were classified as having a dementia disorder. Dementia conversion was predicted from demographic variables, Mini-Mental State Examination score, and olfactory assessments. Self-reported olfactory impairment emerged as an independent predictor of dementia. After adjusting for effects of other predictors, individuals who rated their olfactory sensitivity as ‘‘worse than normal’’ were more likely to convert to dementia than those who reported normal olfactory sensitivity (odds ratio [OR] 52.17; 95% confidence interval [CI] [1.40, 3.37]). Additionally, low scores on an odor identification test also predicted conversion to dementia (OR per 1 point increase50.89; 95% CI [0.81, 0.98]), but these two effects were additive. We suggest that assessing subjective olfactory complaints might supplement other assessments when evaluating the risk of conversion to dementia. Future studies should investigate which combination of olfactory assessments is most useful in predicting dementia conversion.

Addition of pleasant odor can change brain response to unpleasant odor

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Odors influence emotionally mood, alertness, relaxation, memories, and hedonics. Olfaction and emotion have close relationship because they share several limbic regions. Most odors we encounter in daily life are mixture of chemical substances which affect us positively and negatively. Experientially, we use mixed aromatic substances to make unpleasant odor fell like pleasant odor. However, it is unclear how the information of the pleasant and unpleasant components of such mixtures are processed in the human brain. The aim of this study is to investigate the processing of odor mixtures by psychological estimation and functional magnetic resonance imaging (fMRI).

Twenty healthy females underwent fMRI scanning during which they performed a block design task using emotional odors carrying pleasant, unpleasant odor or mixture. We used five odors; one unpleasant odor was Butyric Acid(BA), two pleasant odors were Fruit-Citrus type fragrance(FC) and Casa Blanca type fragrance(CA), two sets of mixtures were the mixture of BA and FC(BA+FC) and BA and CA(BA+CA). fMRI data were analyzed by comparing the images acquired under the conditions described earlier.

The brain regions activated by BA, FC and CA were very different, even though FC and CA were both pleasant. Thus these three odors were processed as different information in brain. In FC condition, significant activation was observed in the anterior frontal lobe, the left parahippocampal gyrus and the left caudate tail. The striatum that includes caudate tail is known to represent reward magnitude. Thus, it is suggested that FC was recognized as a reward. BA+FC produced activation in the superior frontal gyrus and the thalamus where we found no activation in BA or FC only. Whereas BA+CA produced activation in the right inferior frontal gyrus, there was no activation in that area by BA or CA only. Comparing the activation by BA, BA+FC and BA+CA, our data suggested that the activation of left frontal lobe was associated with unpleasant stimuli and that the activation of right frontal lobe was associated with pleasant stimuli.

In conclusion, our findings suggest that each odor is processed differently in the brain. The caudate tail can play an important role in the rewarding effect by olfactory pleasant stimuli. Furthermore, odor hedonics may provide bilateral difference in the frontal activation area.

Changes in smell and taste function in the elderly

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It is known that both smell and taste functions decrease with aging. However, some clinical questions remain, such as the independence of each sensation loss and the correlation between a person's actual sensory loss and awareness of the loss. In this research, we investigated both the olfactory and taste functions of aged subjects together with appetite-related questionnaires and physical abilities.

Sixty healthy volunteers more than 60 years old were recruited in this study (35 females and 25 males, mean age 71.30±5.29 years, range 62-87 years). All participants underwent olfactory, taste and physical examinations. For the taste examination, 60 young adult volunteers were used as a control group. The olfactory examinations included the use of an Open Essence odor identification test, an olfactory visual analogue scale (VAS) and a self-administered odor questionnaire. For taste examinations, a whole mouth threshold test using sweet, salty and umami solutions and a taste VAS were used. Height, body weight, elementary body composition and grasping power were measured.

Out of 60 subjects, 37 showed olfactory dysfunction according to the odor identification test. There was a significant correlation between identification test score and age. In the taste threshold test, the mean score of the aged group was significantly lower compared with the control group for each of the three tastants. There was definitive correlation among the tastant scores for each subject, which suggests that taste dysfunction occurs concurrently across the tastants. The subjects with low olfactory test scores also had low taste test scores; however, the subjects with low taste test scores showed considerable variance in olfactory test scores. There was no definitive correlation between olfactory identification scores, self-administered olfactory questionnaire scores, and the smell-related appetite questionnaire scores. A similar result was noted in the taste test. It appears that many subjects who have olfactory or taste dysfunction might not notice their sensory deterioration.

We use our sense of smell and taste in our daily life; they are important to our QOL as we age. Beyond enjoying foods and beverages, the sense of smell is important for detecting hazards such as leaking gas, fire, and spoiled food. The sense of taste is profoundly concerned with food intake and nutrition support. We believe it is important to notice the state of these chemosensory function deteriorations in the elderly to allow them to live a comfortable life and to help them avoid hazardous events.

Olfactory imprinting in zebrafish: a genetic analysis.

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Imprinting is a learning process during early development that is limited to a short period of time and leads to irreversible changes in behavior. It had been described in a variety of different contexts, such as species recognition, mating, homing, or food preferences. Stimuli triggering the imprinting process can be biotic or environmental cues, such as amino acids and peptides.
Based on behavioural experiments we showed that zebrafish imprint on olfactory cues of kin at day 6 of development. Larvae use the learned cues to identify kin later in life. Imprinting does not occur when larvae experience cues of non-kin, suggesting a genetic predisposition for kin odour.
We demonstrated that MHC genes and peptides are relevant for imprinting and kin recognition. Knowing chemical signals and the genetic basis for imprinting we want to know whether imprinting leads to structural changes in the periphery of the olfactory system.
The aim of our study is to analyze qualitative and quantitative olfactory receptor gene expression in the olfactory epithelium of zebrafish larvae. We address the question whether olfactory imprinting induces differences in the expression pattern of olfactory receptor genes and thus differences in the periphery of the olfactory system in larvae. Six-day old zebrafish larvae are exposed to different olfactory cues, e.g. kin odour. We test the larvae for their olfactory preference in a behavioural assay with a two-channel-choice flume. The gene expression of over 40 olfactory receptor genes and transcription factor genes is analysed before, during, and after olfactory imprinting, using quantitative real-time PCR and next generation sequencing methods. Furthermore, we expose larvae to different amino acids during the sensitive phase of olfactory imprinting, to understand whether imprinting on amino acids induces differential gene expression of olfactory receptor genes in zebrafish larvae.

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Functional characterization of a human olfactory receptor to musk odorants.

Alex Veithen, Françoise Wilkin, Magali Philippeau, Pierre Chatelain

Musk is an olfactory note that is widely used in perfumery and is found in a wide array of products, including fine fragrances, body care, detergents etc. Originating from a natural source (glands of a Himalayan deer), most of the musks used today in the industry follow from chemical synthesis. Four chemical families of compounds with musky notes have been identified: nitro musks, polycyclic musks, macrocyclic musks and alicyclic (or linear) musks. Notwithstanding their commercial success, concerns have been raised against the two first families, due to their potential danger for the human health and for the environment. Their replacement by safer and sustainable surrogates that would preserve their typical hues constitutes a challenge for the musk producers. The understanding of the molecular mechanisms that support the perception of the musky odor would certainly favor the identification of this awaited new generation of musk compounds. In this context, we describe the characterization of OR5AN1, a human olfactory receptor specifically tuned by musk molecules. Using a HEK293 cell-based expression system and luciferase based gene reporter assay, it is shown that the receptor responds, with a very high potency (EC50 below the nanomolar range), to different representatives of the nitro musk family: musk xylene and musk ketone (e.g.). The same receptor is also activated by most of the tested macrocyclic musks, including, among others, cyclopentadecanone, muscone and ethylene brassylate. By contrast, OR5AN1 remained irresponsive to members of the polyclic group, such as galaxolide or tonalide as well as representatives of the alicyclic family, including helvetolide, serenolide and sylkolide. Since none of non-musky molecules tested so far elicited a detectable activation of OR5AN1, it is tempting to speculate that this receptor plays a role in the perception of the musk note. Nevertheless, its insensitivity to 2 of the musk families suggests that other receptors should be involved in the detection of this characteristic scent. Additional candidate receptors for musk are currently studied.

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OR1D2 is a broadly tuned human olfactory receptor

Alex Veithen, Françoise Wilin, Magali Philippeau, Pierre Chatelain

OR1D2 is one of the first deorphanized human olfactory receptors. It was initially found to respond to bourgeonal and to a limited series of analogs including canthoxal, lillial and florazone. The main structural feature shared by these activators is a benzene ring with a short lateral chain ended by an aldehyde. At ChemCom, the receptor was included in a series of screening campaigns that aimed at identifying agonists for human ORs among libraries of 200 to 400 selected odorant molecules. These libraries have been designed to cover a large diversity of both chemical structures and organoleptic properties. The screenings were performed using a HEK293 cell-based expression system and a CRE-Luciferase gene reporter assay. From these different campaigns and subsequent validations, we identified 90 agonists, including aliphatic, aromatic, cyclic or linear compounds. No shared structural characteristic emerges from the comparison of the different activators that can be alcohols, aldehyde, esters, lactones, etc. Likewise, the different agonists are not linked by a common organoleptic characteristic or note. Both pleasant and unpleasant odors are represented in the list of OR1D2 ligands, though the most potent activators are generally perceived as having pleasant scent.

Our study shows that this receptor belongs to the intriguing category of broadly tuned receptors for which the role in the discrimination of odors remains to be understood. Since OR1D2 is also expressed in spermatozoa, its ability to respond to a large number of ligands could shed a new light on its function in sperm chemotactism.

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Responses to Domestic Cat Chemical Signals are Modulated by Early Olfactory Experience in the House Mouse

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Chemosensory detection is an important aspect of predator avoidance strategy. Finding of universal carnivore signal (Ferrero et al., 2011) does not explain why only odors from natural predators produce profound effects on the behavior and the neuroendocrine system of prey. It suggests the ability of prey to distinguish predator species by odors. Domestic cat is the most specialized predator to the house mouse. We examined the influence of the species specific compound from the cat urine L-felinine on the reproduction and investigatory behavior of mice in comparison with the cat urine and how the response to cat odors could be modulated by early olfactory experience. Two basic approaches were used: behavioral and endocrinological. Olfactory thresholds to cat urine and L-felinine were measured with an automated olfactometer (Knosys, USA). Number of newborn pups and sex ratio was recorded. Plasma corticosterone and fecal corticosterone metabolites were monitored non-invasively using ELISA technique. Patterns of investigatory activity and avoidance behavior were analyzed using an open field paradigm. Percent of animals with block of pregnancy was significantly higher (n=26, $P<0.001$) in adult mice exposed to L-felinine (0.05%). Exposure of adult mice to L-felinine also affected sex ratio (n=26, $p<0.001$) in favor of males (Voznessenskaya, 2014). The observed effects could be explained in part by long lasting elevation of corticosterone under L-felinine (0.05%) exposures (n=13, $p<0.001$). Exposures of mice to cat urine or L-felinine during critical period for odor sensitization (14-28 days after birth) significantly lowered the olfactory thresholds to cat urine or L-Feinine (n=10, $p<0.001$) which is adaptive for the predator detection. Early ontogenetic exposures also decreased patterns of passive-avoidance behavior (n=22, $p< 0.05$) in response to cat urine/feinine in the open field and significantly increased investigatory activity as indicated by elevated number of mountings ($p< 0.05$ n= 22) and elevated number of the hole investigations ($p< 0.05$ n=22). At the same time corticosterone response to domestic cat chemical signals stayed unchanged indicating the innate nature of the response.

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Deorphanization of human olfactory receptors

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ChemCom progresses towards its objective of deorphanizing the whole repertoire of human olfactory receptors (ORs). Relying on (i) its proprietary technology, (ii) libraries of thousands of odorant compounds and (iii) an efficient screening system, ChemCom is currently identifying and characterizing new modulating molecules (enhancers or blockers) and novel odorant compounds for the whole range of human ORs. The profiling of expression OR gene in the whole olfactory mucosa and the analysis of its distribution in the human population recently achieved at ChemCom, provides a unique opportunity to select among 273 frequently expressed ORs in view of a systematic deorphanization (Verbeurgt et al, 2014). At ChemCom, more than 100 ORs have been robustly and specifically deorphanized. The number of odorant molecules quoted as agonists for these ORs largely exceeds 1,000, with an average of 19 agonists per OR and a maximum of 216 activators for a single OR. By contrast, one molecule activates 2 different ORs in average and a number of 13 ORs activated by the same molecule has been observed as the upper limit. So far, only 5% of the ORs were found to respond to more than 80 agonists from different chemical and organoleptic properties and are therefore considered as broadly tuned. The remaining ORs are considered as moderately (>5 agonists per OR; 49%) or narrowly (≤5 agonists per OR; 46%) tuned. Interestingly, most of the ORs deorphanized to date belong to the expressed OR gene set. Indeed, among the 47 deorphanized receptors reported in publications, 43 are found to be expressed. Here, we present a data sheet for twelve highly expressed and deorphanized ORs which respond to representative molecules such as Musks, Ionone, Carvone, Sotolon, Lactone, Geraniol, Vanillin, Androstenone, Pyrazines, Thiols, Carboxylic acids,… Besides the results presented in this poster, Chemcom holds confidential data on ORs responding to pleasant odor of interest (e.g. amber, marine, green, floral, fruity, spicy, anisic…) and also to typical malodors (e.g. sweat, mildew, animal, fishy, sulfurous, beverage and food off-notes,…).

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Analysis of whole nerve responses in the chorda tympani nerve from mice after daily intake of sweet solution

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It is unknown whether habitual intake of sweet beverages causes change of taste sensitivity. To examine taste sensitivity after daily intake of sweet solution, we recorded whole nerve responses to NaCl, KCl, HCl, sucrose, SC45647, saccharin, glucose, glycine, quinine HCl, monopotassium glutamate (MPG) with and without 0.5 mM inosine monophosphate (IMP) in the chorda tympani (CT) nerve after mice were given 2 mM saccharin for 3, 7 and 14 days instead of water. Responses to sucrose, SC45647 and other sweet compounds were significantly reduced when C56BL/6J mice (22 – 25g B.W.) were given 2 mM saccharin for 7 and 14 days as compare with control mice that were given water. Same results were obtained when another group of mice were given 0.3 mM SC45647 for 7 days instead of 2 mM saccharin. Further we recorded whole nerve responses from CT of diet-induced obesity (DIO) (13-week old males with 9 weeks on high fat diet) mice after mice were given 2 mM saccharin for 7 days. Responses to sucrose, SC45647 and other sweet compounds were significantly increased as compare with control DIO mice that were given water. These data suggests that responses to sweet compounds decreases in the CT of C56BL/6J mice (22 – 25g B.W.) and increases in the CT of DIO mice by daily intake of sweet solution.

Spontaneous and odor-evoked firing activity in mouse olfactory sensory neurons expressing the I7 odorant receptor

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Odorant receptors located in the cilia of vertebrate olfactory sensory neurons bind specific odorant molecules and activate a transduction cascade, which leads to depolarization and the generation of action potentials. We measured firing properties of olfactory sensory neurons in acute coronal slices of the main olfactory epithelium from P0-P4 mice, using extracellular loose-patch recordings from dendritic knobs. This technique allows the recording of both spontaneous and odor-evoked activity minimizing any disturbance due to membrane damage or modification of the cytoplasm composition. To characterize odor-evoked activity patterns of olfactory sensory neurons expressing a defined odorant receptor, we used gene-targeted mice in which the green fluorescent protein (GFP) is co-expressed with the odorant receptor I7. Since it is well known that the mouse I7 receptor is well activated by the heptanal, we stimulated the green fluorescent neurons with this odorant. We varied the stimulus by applying heptanal pulses of different durations or using different heptanal concentrations with a multibarrel pipette. Heptanal stimulation induced two types of responses: a brief burst of action potentials, or an initial burst followed by a silencing period and a rebound activity of action potentials. Brief bursts were usually evoked by less intense stimulations, while the more complex response pattern was evoked by more intense stimulations. Most I7-GFP neurons fired spontaneously with quite heterogeneous spontaneous firing frequencies. Our results are similar to those of previous reports obtained with recordings with the suction electrode from isolated I7-GFP neurons (Reisert, 2010), or with cell-attached recordings from I7-GFP neurons in the intact olfactory epithelium preparation (Connelly et al., 2013) from adult mice. Thus, the coronal slice preparation from pups mice is suitable for studies aiming to the characterization of the physiological role of proteins involved in the transduction cascade or in the modulation of the firing activity of olfactory sensory neurons.

We thank Peter Mombaerts for providing the I7-GFP mouse line.
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Session 2 P1

Nosewitness identification: lineup test performance following emotion (crime) and neutral videos

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Every individual has a unique body odor (BO), similar to a fingerprint. In forensic research, identification of culprit BOs has been performed by trained dogs, but not by humans. We introduce the concept of *nosewitness identification* and present the first experimental results on BO memory in witness situations involving violent crimes. Two experiments indicated that BO associated with male characters in authentic videos could later be identified in BO line-up tests well above chance. Moreover, culprit BO in authentic and emotional crime videos could be identified considerably better than the BO of a male person in neutral videos. This indicates that nosewitness identification, in contrast to typical eyewitness studies, benefits from emotional encoding. Altogether, the study testifies to the virtue of body odor as a cue to identify individuals observed under negative emotion.

Pulse Width Modulation applied to olfactory stimulation for modifying its intensity

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For most olfactometers described in the literature, adjusting olfactory stimulation intensity involves modifying the dilution of the odorant in a neutral solution (water, mineral, oil, etc.), the dilution of the odorant air in neutral air flow, or the surface of the odorant in contact with air flow. So, for the above-mentioned devices, manual intervention is necessary for adjusting concentration. We present in this poster a method of controlling odorant concentration via a computer which can be implemented on even the most dynamic olfactometers. This technique is Pulse Width Modulation (PWM), commonly used in electronic or electrical engineering. We have applied it to odor delivery.

PWM, when applied to odor delivery, comprises an alternative presentation of odorant air and clean air at a high frequency. The cycle period (odor presentation and rest) is 150 ms. In order to modify odorant concentration at system output, the duty cycle is modified: the ratio between the odorant period and clean air presentation during a cycle. Chromatography measurements show that this method offers a range of concentration from 30% to 100% (continuous presentation of odor).

Proof of principle is provided via a psychophysical experiment. Three odors (isoamyl acetate, butanol and pyridine) were presented to fourteen subjects. Each odor was delivered with six values of duty cycle: from 10 % to 100% with steps of 18%. After each stimulation, the subjects were asked to estimate intensity of the stimulus on a 10 point scale, ranging from 0 (undetectable) to 9 (very strong).

The mean of intensities range from 3.6 to 5.4 for butanol, from 3.6 to 6.3 for isoamyl acetate and from 3.8 to 6.7 for pyridine. Paired Student t-tests showed that changing duty cycle can significantly modify the intensity of stimulation.

Increasing the detectability of olfactory event-related potentials – measurement of olfactory event-related-potentials with a short inter-stimulus interval

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Objectives: Although the measurement of olfactory event-related potentials (OERP) is an established method to objectively assess olfactory function, there is still room for improvement. Aim of this study was to increase the detectability / improve the signal-to-noise ratio.

Material & Methods: One hundred-two participants (51 female, 51 male) with a mean age of 44.3±17.5 years were included in the study. The participants were normosmic, hyposmic and functionally anosmic as ascertained by means of the "Sniffin' Sticks" test battery. OERPs in response to phenyl ethyl alcohol were measured separately for the left and right nostrils. The inter-stimulus interval (ISI) was set to either 30 or 10 seconds with 16 and 60 stimuli repetitions, respectively. OERPs were recorded from five electrodes (Cz, Fz, Pz, C3 and C4). In addition to the signal-to-noise ratio amplitudes and latencies were measured for OERP components N1 and P2.

Results: When the ISI was set to 30 seconds, amplitudes of N1 and P2 were larger in comparison to amplitudes obtained with 10 seconds ISI (p=0.001). Although the signal-to-noise ratio was not different between the two ISI conditions, in normosmic subjects it was more likely to obtain an OERP with the shorter ISI (both nostrils together 91 vs. 86%, n.s.). Interestingly in functionally anosmic patients it was more likely to obtain an OERP with the longer ISI (both nostrils together 35vs. 23%, n.s.). Again this difference did not reach the level of significance.

Conclusion: Although not statistically significant, the detectability for OERP in normosmic adults was slightly higher with a 10 second ISI compared to a 30 seconds ISI in contrast to anosmic patients.

Session 2 P4

Biophysical and functional characterisation of the human olfactory receptor OR1A1 expressed in a mammalian inducible cell line

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Researches on olfactory receptor (OR) features revealed that the OR family includes members that are able to respond to a large set of odorants as well as members that are activated by a relatively small number of related odorants. In order to fully understand how ORs recognize their ligands, it is crucial to perform biophysical and biochemical studies, which will provide fundamental insights into OR function at the molecular level as well as structural information. To enable biophysical and functional characterization, we used a recently developed mammalian expression system, the tetracycline-inducible HEK293S cells lacking N-acetylglucosaminyltransferase I (GnTI$^-$), which is suitable for large-scale G-protein coupled receptors production. We took advantage of this recent advance to produce and purify the human OR OR1A1 (hOR1A1) that has been shown to be activated more specifically by odorant molecules such as cetones and aldehydes. First of all, a HEK293S GnTI$^-$ cell line that stably expresses the synthetically engineered hOR1A1 gene at high-levels was selected. After solubilization of membrane proteins using fos-choline-14, a two-step purification method (immunoaffinity purification using immobilized anti-Flag-tag monoclonal antibody and gel filtration), was employed to purify the receptor. SDS-PAGE analysis and western-blot analysis revealed that hOR1A1 was pure. The total amount of purified receptor was approximately 25 µg per 175 mm plate. The level of expression is 10 fold higher that those reported for hOR17-4. Secondary structure analysis using circular dichroism demonstrated that the purified hOR1A1 receptor was properly refolded. Analysis of the protein using online size-exclusion chromatography coupled with multiangle light scattering (SEC-MALS) indicated that monomeric and dimeric forms of hOR1A1 are both present. Intrinsic tryptophan fluorescence spectroscopy was used to investigate hOR1A1 binding properties. These experiments revealed that hOR1A1 is able to bind dihydrojasmone with an affinity in the micromolar range, in agreement with previous data obtained through functional cellular assays. This approach will contribute to enhance our knowledge regarding structural and functional properties of hOR1A1 and more generally, can help understand molecular mechanisms of olfaction.

Session 2 P5

In vivo optogenetic analysis of interglomerular lateral interactions

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Glomeruli are the functional units of olfactory processing. Understanding how neuronal activity in a single glomerular unit propagates through olfactory bulb circuits has been challenging, in part because odors activate many glomeruli. We expressed channelrhodopsin-2 in olfactory sensory neurons that express a defined odorant receptor, thus allowing us to combine optogenetic control and calcium imaging of a single target glomerulus *in vivo* . Low intensity light stimulation of channelrhodopsin-2 expressing sensory neurons produced unimodal excitatory calcium responses in the target glomerulus and in hundreds of closely-associated juxtaglomerular neurons. Inhibition was observed rarely, in neurons that were irregularly distributed around the target glomerulus. High intensity light stimulation caused neural excitation to spread to neighboring glomeruli. These signals were accompanied by activation of GAD65-expressing juxtaglomerular neurons and likely reflect *interglomerular* lateral inhibition. Our data show that glomeruli modify incoming olfactory information via lateral inhibition *in vivo*.

Session 2 P6

Haemodialysis in patients with chronic renal failure increases the wanting for foods rich in proteins

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Introduction/Objectives
Protein-energy malnutrition is a risk factor for mortality in patients with renal failure (RF). Changes in food preferences induced by haemodialysis are not known. The objective of the study was to investigate in these patients the components of the reward system (wanting and liking) for foods rich in proteins, carbohydrates and lipids.

Methods
Twenty-five patients with RF were evaluated the day and 24h before or after their haemodialysis. They were compared with 25 matched healthy subjects (sex, age and BMI). Sensations of hunger, wanting (presentation of 16 pictures representing the 3 classes of macronutrient), olfactory liking (presentation of 6 foods representing the 3 macronutrient) were evaluated at 7am and 11am. Plasmatic ghrelin, leptin, insulin and amino-acids were evaluated simultaneously.

Results
At 7am and 11am, hunger sensation of patients did not differ from that of healthy subjects. The wanting for protein-rich foods, similar at 7am, was lower than that of controls at 11am (3.9 ± 2.4 vs 5.8 ± 2.5, $P < 0.001$) but was restored to the same level as the healthy subjects after haemodialysis ($5.0 + / -2.3$, $P < 0.01$). The wanting for fatty-rich and carbohydrate-rich foods and food liking for the 3 classes of macronutrient did not differ between groups. In patients with RF only, change in plasma amino-acids was negatively correlated ($P < 0.01$) with the change in wanting for protein rich-foods. Indeed, glutamine, alanine, citrulline, phenylalanine, ornithine and proline decreased after the haemodialysis while the wanting for protein-rich foods increased. In contrast, changes in plasmatic ghrelin, leptin, insulin did not correlated with the changes in wanting for protein-rich foods.

Discussion/Conclusion
Patients with RF increase their wanting for the protein-rich foods immediately after haemodialysis. This result can be explained by a decrease of several plasmatic amino acids induced by haemodialysis. Therefore, this study suggests that an increase in protein intake immediately after haemodialysis could be beneficial for patients with RF who frequently present protein-energy deficiency.

Development of mutant Odorant Binding Proteins (OBPs) for Security Applications: Detection of Explosives and Drugs

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Using *in silico* mutagenesis and docking screening techniques the binding pocket of the wild type (WT) mosquito *Anopheles gambiae* Odorant Binding Protein OBP1 (AgamOBP1) was modified to produce variants with enhanced affinities to target explosives and drugs. 31 binding pocket residues that have direct contact with the selected ligands were identified. Single amino acid substitution at each of the 31 residue positions using all 20 amino acids was studied. Analysing the stability of the resulting mutant using rigidity analysis 13 of the binding pocket residues were found to be feasible for mutation. Further analysis of the mutant stability using energy analysis, via direct amino acid substitutions identified 28 stable mutations possible at six different positions. Each of these 28 mutants was docked *in silico* with a range of target explosives and drugs. Analysis of the docking outcome data indicated that 17 mutant variants were likely to have stronger binding affinities towards the ligands compared to the WT. We then expressed four AgamOBP1 mutants plus WT and determined their binding properties towards the target explosives and drugs in solution using fluorescence based competitive binding assays. Some of these mutants indeed show very high affinity to the target analytes compared to WT protein. One example is the mutant AgamOBP1_S82P, this has a dissociation constant (KD) of 0.48 µM towards cocaine compared to 11. 57 µM for WT and KDs of, 0.34 µM for Atropine, 0.28 µM for THC and 0.39 µM for MDMA (Ecstasy), compared to KDs of 4.81 µM for Atropine, 3.59 µM for THC and 8.58 µM for MDMA (Ecstasy) for the WT. For target explosives the mutant S82P has the KDs of 1.36 µM, 0.39 µM, 0.57 µM and 0.13 µM towards, TNT; 2,4-DNT; 2,6-DNT; and NH_4NO_3 respectively, compared to WT with the KDs of 3.95 µM, 3.15 µM , 2.09 µM, and 6.3 µM towards, TNT; 2,4-DNT; 2,6-DNT and NH_4NO_3 respectively. We then immobilised these OBPs onto quartz crystal microbalances (QCM) and we have found that they can sensitively detect the target analytes in vapour form as well. We have demonstrated that *in silico* mutagenesis and docking screening techniques are powerful tools for designing variants of AgamOBP1 that are stable and display enhanced affinities towards the target analytes.

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Session 2 P8

Bisphenol A from drinking water affects taste preferences and oral homeostasis in male rat.

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Context : Bisphenol A (BPA) is used to made polycarbonate plastics and epoxy resins for manufacturing food packaging, medical consumables and dental sealants. So, it could reach high levels in saliva. Because of estrogen-like properties, we suspected that BPA could affect mouth estrogen sensitive targets. This work aims to establish the effects of BPA on gustatory preferences and submandibular salivary glands that are both sex hormone-regulated.

Experiment: A dose-response study (5 dosed-groups (n=12) has been performed on adult male Wistar rats by giving BPA for 6 weeks via drinking water solutions in order to have 0.5 µg/kg, 50 µg/kg, 5 mg/kg and 12.5 mg/kg daily dose exposures. On the last week of treatment, sweet, salt and fat preferences were evaluated for 3 days using the two-bottle test method. Rats were then killed for salivary gland removing and mRNA salivary proteins biomarkers.

Results : BPA decreased sweet and fat preference according to a U-curve dose-response and increase salt preference in a linear dose response ($p<0.05$). A strong mouth dryness was observed from the lower BPA dose ($p<0.01$) coupled to an histological effect on acini structures and mRNA salivary protein expression in submandibular.

Conclusion: Mouth dryness and disrupted taste preferences induced by BPA drinking could be the result of a disrupting effect on salivary glands. BPA induced oral symptoms that are similar to deleterious phenomena observed in mouth homeostasis and taste lost from elderly patients.

Eugenol and carvacrol elicit oral irritation and enhance warmth and heat pain via effects on primary sensory neurons in rats

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Eugenol and carvacrol from clove and oregano, respectively, are agonists of TRPV3 which is implicated in transduction of warmth and possibly heat pain. We investigated the temporal dynamics of lingual irritation elicited by eugenol and carvacrol, and their effects on thermosensitivity, in humans. We additionally assessed if these agents sensitize thermally-evoked responses of primary sensory trigeminal ganglion (TG) neurons and second-order neurons in trigeminal subnucleus caudalis (Vc). In the human psychophysical experiments, epilingual application of eugenol (600 mM) or carvacrol (50 mM) elicited irritation that decreased significantly across repeated applications (desensitization). To assess effects of eugenol and carvacrol on lingual warmth and heat pain, either chemical was applied unilaterally, followed 0, 0.5, 5 and 10 min later by bilateral application of a warm (44°C) or painful (49°C) thermal stimulus. Using a 2-alternative forced-choice design, a significant proportion of subjects chose the eugenol- or carvacrol-treated side to be warmer or more painful, respectively, indicating enhancement of warmth and heat pain. This effect was transient and was corroborated by higher intensity ratings of warmth or pain on the treated side. To assess if these thermal enhancement effects occur peripherally and/or centrally in Vc, we performed two sets of animal experiments: (a) in vitro calcium imaging of cultured rat TG and dorsal root ganglion (DRG) neuronal responses and (b) in vivo recording of Vc neuronal responses to lingual stimulation in anesthetized rats. For calcium imaging, eugenol (200 µM), carvacrol (100 µM), or heated Ringers solution were separately applied to cultured TG and DRG cells loaded with Fura-2. A thermocouple monitored bath temperature near the cells. Overall, 22.5% of TG and DRG cells responded to a warm (39°C) stimulus and 48.6% responded to a noxious (42°C) heat stimulus. Responses to repeated thermal stimuli were stable. When preceded by application of eugenol or carvacrol, responses to the second 39°C or 42°C stimulus were enhanced. In pentobarbital-anesthetized rats we isolated lingual heat-sensitive Vc neurons. Both eugenol and carvacrol briefly enhanced noxious heat-evoked responses. These results indicate that eugenol and carvacrol peripherally enhance responses of trigeminal and spinal sensory nerve endings to innocuous warming and noxious heat, thus accounting for the effects of these agents to enhance heat-evoked responses of Vc neurons and to enhance perceived lingual warmth and heat pain in humans.

Olfactory function in the Down syndrome

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The Down syndrome (DS) is one of the most common congenital chromosome disorders in humans. It is a particular combination of different phenotypic features that includes a characteristic *facies* and mental retardation (from mild to severe). This syndrome shows Alzheimer-like neuropathological findings and clinical features of deterioration similar to those typical of Alzheimer disease. For example, DS patients over the age of forty years show senile plaques and neurofibrillary tangles in the brain. It is well known that Alzheimer disease is characterized by olfactory impairment. Because of the neuropathological similarities between DS and Alzheimer disease, it was hypothesized that olfactory impairment would also be present in DS. In the last thirty years, some works investigated olfactory function in a variable number of DS patients of different ages using different methods. These studies mainly assessed odour threshold or identification, or olfactory memory. Only one study considered olfactory discrimination performance. While all studies reported some olfactory impairment in DS subjects, a comprehensive evaluation of olfactory function in the DS is lacking. In this work, we assessed olfactory function in male (M) and female (F) DS patients (n=44; M=25, F=19) over a wide range of age (age range, x-y years; mean age 30.3±1.63y) in comparison with euploid healthy control subjects (n=54; M=27, F=22, mean age 26.3±1.51y). We used a standardized test (Sniffin' Sticks Extended test, Burghart Company, Germany) to measure olfactory threshold, discrimination, and identification performance; results of the three subtests are presented as a composite global score defined as "TDI score". This procedure has been validated on more than 3000 subjects and it is widely used in Europe but, to our knowledge, never applied in DS subjects.Data were analyzed using one-way ANOVA and the Pearson (r) correlation coefficient. Results showed that DS subjects have lower threshold, discrimination, and identification performance than controls (3.0±2.53 vs. 8.4±2.86; 5.7±1.91 vs. 12.9±1.76; 7.6±2.66 vs. 14.0±1.32, respectively; $p<0.001$ for all); accordingly, the TDI score was 11.3±3.11 in DS subjects and 35.2±3.75 in controls ($p<0.001$). Such differences persisted after adjusting data for age or sex. The results obtained so far show that olfactory function is overall impaired in DS subjects . The relationship between level of cognitive functioning and olfactory performance is currently being explored.

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Impact of an olfactory priming on food intake: a potential intervention tool in an Alzheimer population

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Our previous studies have revealed an impact of olfactory priming on food choices behaviour in "healthy" adults (Gaillet, Sulmont-Rossé, Issanchou, Chabanet, Chambaron, 2013; 2014). These studies demonstrated that non-attentively perceived fruity odours could impact food choice, guiding participants toward items containing more fruit and/or vegetables. To extend this research, we aim to study the impact of spreading a food odour before lunch (i.e., olfactory priming) on subsequent food intake and eating behaviour of elderly patients suffering of Alzheimer's or a related disease. A characteristic of this disease is that explicit memory (declarative) of these patients is impaired whereas their implicit memory (non declarative) remains relatively preserved. As a significant part of consumers' food choices seems to be influenced by non-conscious processes, it is particularly interesting to study the impact of olfactory priming with Alzheimer patients.
Thirty-two residents (> 75 yo) from three Alzheimer's Units were included in the study. They participated in a 'control' lunch and a 'primed' lunch, for which a meat odour was diffused in the dining room 15 minutes before the arrival of the meal tray (olfactory priming). Two measures were carried out for each participant: food intake measurement and behavioural assessment (interest to the meal, staying sitting at table, interaction…). This procedure was replicated: participants completed a second control and primed lunch.
Results of the first replication showed a significant effect of olfactory priming, with a 25% increase in meat and vegetable consumption compared to the control condition. Behavioural measurements also show a significant increase of resident's interest toward the meal in the primed lunch. However, these effects disappeared at the second replicate.
Considering the results observed for the first replication, it is worth to conduct other experiments to check if such an effect can be replicated and to investigate why the significant effect of olfactory priming observed in the first replicate faded in the second. It is a key point for demonstrating that olfactory priming could be a potential lever to restore desire to eat in elderly patients suffering of Alzheimer's and thus helping to fight against malnutrition and improve the quality of life of this specific elderly population.

Human Visual Evoked Potentials are Enhanced by Long-Term Visuo-Gustatory Conditioning

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Aim: The taste of food and drink can be recognized from its visual appearance on the basis of memorized visuo-gustatory associations. This raises a question whether visual processes, evoked in the human brain by the sight (conditioned stimulus, CS), are altered by association with a taste (unconditioned stimulus, US). The question was elucidated using high-density EEG scanning.

Methods: Before image-taste association, subjects were presented with two unfamiliar images. Visual evoked potentials (VEPs) were recorded for each of them. In a subsequent training session, one image was associated with the taste of an appetitive apple juice, and the other with an aversive version of the same juice containing NaCl, glutamate and ferro-sulphate. One day later, VEPs evoked by the same images were recorded again. Each image had a balanced use between subjects as CS presented before either appetitive or aversive US.

Results: Two prominent VEPs peaks were examined: N2 and P3. The average peak delays for N2 were recorded in 20 electrodes placed over posterior visual cortex areas. Before appetitive conditioning, the average N2 peak delay was 165.4 ± 2.0 ms (mean ± sem, n=7) compared to 157.8 ± 2.0 ms after training ($p<0.0001$); for aversive conditioning: 167.6 ± 1.6 ms before and 160.3 ± 1.7 after ($p<0.0001$).

The average N2-P3 amplitudes were examined for the same 20 electrodes. For appetitive conditioning before training, amplitudes were 10.8 ± 0.4 µV versus 12.8 ± 0.5 µV after; for aversive conditioning: 10.5 ± 0.4 µV before and 12.4 ± 0.5 µV after ($p<0.0001$ for both). Analysis of the power of VEPs in the time-frequency space (performed after Fast Fourier Transformation) revealed that the main power of the VEP waves fell between 4 and 8 Hz ("theta frequency range"). Theta power for appetitive conditioning was 3.3 ± 0.7 μV^2 before and 8.1 ± 0.7 μV^2 after; for aversive conditioning: 3.5 ± 0.3 μV^2 before and 6.7 ± 0.6 μV^2 after ($p<0.001$ for both). No quantitative differences were observed between the effects of appetitive and aversive conditioning.

Conclusions: One day after image-taste conditioning, visual CNS-processing was changed: (1) the processing speed was enhanced, (2) image-induced VEP amplitudes were augmented leading to (3) enhanced visually induced power in the 4-8 Hz frequency band.

Compensatory Plasticity in the Olfactory Epithelium: Timing and Reversibility

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Like other biological systems, olfaction responds homeostatically to perturbations, including chronic fluctuations in the stimulus milieu. This phenomenon, referred to as compensatory plasticity, appears to be implemented at multiple levels of the olfactory system; however, it has been most thoroughly studied at the periphery (Coppola, 2012). In the olfactory epithelium, chronic stimulus deprivation or enrichment trigger opposite changes in the transcription/translation of proteins involved in odor transduction. These responses, at the molecular level, to odor environment manipulation are thought to underlie the modulation of receptor neuron gain observed physiologically and, at least in part, odor threshold modulation observed behaviorally. Until now, olfactory compensatory plasticity has been studied, almost exclusively, in developing animals. It is unknown whether, like in other sensory systems, this plasticity has a 'critical' period and is irreversible. Here we study unilateral odor deprivation in adult mice using nasal plugs to eliminate nasal airflow unilaterally. Plugs were placed in one nostril for two to six weeks after which electroolfactograms (EOGs) were recorded from the olfactory epithelium of the occluded and open sides of the nasal cavity. The stimuli were isoamy acetate, carvone, and eucalyptol, delivered at the nominal concentration of one part per thousand. Untreated animals served as negative controls and adult mice that had undergone standard neonatal naris occlusion by cautery served as positive controls. Mean EOG amplitudes from plugged mice were significantly greater on the occluded side than the open side of the nasal cavity. The duration of plugging did not affect the results, suggesting that plasticity occurs within two weeks. Moreover, EOG amplitude differences between the open and occluded sides of the nasal cavity in plugged animals were comparable to those seen in positive controls (Waggener & Coppola, 2007). In another group of adult mice, plugs were allowed to stay in place for four weeks and were then removed for two weeks. After this recovery period, EOG mean amplitudes were not significantly different between the always-open and previously-plugged sides of the nasal cavity suggesting that compensatory plasticity in the olfactory mucosa is reversible. Taken together, our results suggest that the olfactory epithelium has an age-independent and reversible ability to rapidly adjust its sensitivity, a process that would maximize the useful odor information reaching the brain in a changing environment.

Elemental abilities of newborn rabbits facing multicomponent odour mixtures

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Most studies comparing olfactory abilities between animal models and humans assess odour thresholds. However, discrimination skills, namely the ability to extract information from complex odour mixture, are also crucial since the organisms are daily confronted to a complex chemical environment. The elemental abilities of adult rats and humans seem limited and decrease when odour mixtures include more than 4 components. Here, we questioned these putative limits in a young mammal, the newborn rabbit. Previous results have shown that newborn rabbits perceive the 6-component RC mixture (a mixture configurally perceived in human adults) in a weak configural manner. Thus, after conditioning to one single odorant of RC by associative pairing with the mammary pheromone (MP), they did not generalize their conditioned behavioural response to the mixture. However, they respond to all the single odorants after learning the mixture, demonstrating their ability to discriminate each odorant from the other five even in presence of the RC configuration. In the present study, we determined the newborn rabbits' perception of more complex mixtures including 13 to 20 odorants with the aim to test their elemental limits. To do so, 375 1-day old rabbits were either conditioned to one element and tested to the mixture or conditioned to the mixture and tested to the elements. The results revealed that more than 70% of pups responded to the 13-odorant mixture after learning one element, whatever its quality. Moreover, after learning the mixture, >88% responded to the elements. Thus, the pups seem perceiving this 13-odorant mixture in a pure elemental manner. In contrast, after being conditioned to one element, they did not generalize systematically their response to the 20-odorant mixture, suggesting a decrease in their elemental abilities. Nevertheless, even after being conditioned to the 20-odorant mixture, more than 60% of pups responded to each element of the mixture (but not to control odorants), suggesting a preserved ability to detect and discriminate elements during MP-induced conditioning. Taken together, these results show that odour mixture perception in newborn rabbits remains in part elemental, for certain complex mixtures, although they contain 13 or 20 components. This suggests that rabbit pups present higher elemental abilities than adult rodents and humans. The putative mechanisms underpinning these striking abilities will be discussed (e.g., distinct perception during conditioning *vs.* retention, associative strength of the MP, influence of the development).

Characterization of the pig sweet taste receptor by heterologous expression.

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The ability to taste sweet compounds is an important quality for evaluating the nutritious content of food. There are, however, differences in sweet taste detection amongst various species such as humans, mice and pigs. While the aforementioned species show clear preferences for natural sugars such as glucose, fructose and sucrose, not all artificial sweeteners that taste sweet to humans are detected by rodents or pigs. To date, the characterization of sweet taste in pigs has only been assessed using preference tests.

Here we report the sequencing and cloning of the Tas1r2/Tas1r3 subunits of the sweet taste receptor of the domestic pig (*Sus scrofa*) and its characterization by means of a heterologous expression system based on HEK293PEAKrapid Gα15 cells. Using our validated cell based expression system, we show that the observed preference of pigs towards the artificial sweeteners, saccharin, acesulfame K, sucralose, stevioside and alitame, is mediated by pTas1r2/pTas1r3. Additionally, HEK293PEAKrapid Gα15 cells expressing pTas1r2/pTas1r3 also respond to D-tryptophan (a sweet amino acid) and xylitol (a sugar alcohol), both of which have been shown to elicit a taste preference in pigs. The work reported here will provide a platform for the identification of binding motifs for artificial sweeteners in pTas1r2/pTas1r3, underlining potential differences with those in human. Furthermore, it will allow for comparison of activation ranges of these and other sweeteners between pig and human Tas1r2/Tas1r3.

Characterization of 5' and 3'UTR sequence isoforms of odorant receptor mRNAs in the mouse olfactory system.

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Olfactory sensory neurons (OSNs) project from the olfactory epithelium to stereotyped targets within the olfactory bulb such that OSNs that express the same odorant receptor (OR) project to specific glomeruli. Thus, ORs have at least two main functions in olfactory sensory neurons in mice: besides their conventional role in the detection and transduction of odorant molecules, they also play a critical role in controlling the sorting and coalescence into glomeruli of axons of the same OR identity. Indeed, OR proteins are present in distal parts of OSN axons, where their homotypic fasciculation is probably initiated. We previously demonstrated that axonal OR mRNAs are associated to polysomes in axons, indicating that axonal OR proteins may have a local origin. Furthermore, we observed that the localization and local translation of OR mRNAs in axons are developmentally regulated. So far, the molecular mechanisms involved in these regulatory processes are unknown, and we are now characterizing OR mRNAs, looking for potential cis-regulatory sequences. We first focused on 3' untranslated regions (3'UTRs), since it has been reported in other systems that long 3'UTR isoforms of mRNAs are specifically addressed to axons, where they are locally translated. Studying a couple of ORs, we demonstrated the expression in the olfactory epithelium of multiple isoforms of these mRNAs, due to alternative polyadenylation. Moreover, we are interested in 2 other mechanisms providing multiple isoforms for OR mRNAs: alternative splicing / intron retention and alternative transcription start sites, creating 5'UTR variations in those mRNAs. Combining RT-qPCR, *in situ* hybridization and northern blot analyses, we are investigating the relative expression and localization of these isoforms in the OSNs. In future work, understanding the regulation of axonal localization and local translation of OR mRNAs will allow to further investigate the role of this local translation in the formation and maintenance of the olfactory primary map.

Sensitivity to human body odor compounds

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Human body odor highly varies as a function of metabolic factors, related to diet and health for example (Havlicek and Lenochova, 2006; Olsson et al., 2014). It also possesses more stable characteristics that are function of genetic relatedness (twin study: Roberts et al., 2005) and that differentiate between the sexes (Bird and Gower, 1981; Troccaz et al., 2009). Attempts have been conducted to identify the odorous compounds involved in these variable and constant qualities, and in the repulsive/attractive properties of body odor. Unfortunately, research has focused almost exclusively on a particular category of compounds: androgen steroids (androstenone and androstadienone). Other chemical compounds that are very typical of human body odor, such as carboxylic acids and sulfanyl alcohols, should be investigated though. The aim of this ongoing study is to investigate quantitative and qualitative aspects of olfactory perception of such volatile compounds, that are present in sex-specific proportions in human sweat. The following molecules are used: 3-hydroxy-3-methylhexanoic acid (HMHA) and 3-methyl-3-sulfanylhexan-1-ol (MSH) (see Troccaz et al., 2009), but also the more commonly studied androstenone (AND) and a control odorant. Adult men and women (age range 18-40) are asked to perform a threshold detection task for the four molecules, using a staircase ascending method with 16 concentrations of each odorant and a four-alternative forced-choice method. After each threshold measurement, participants are asked to evaluate familiarity, pleasantness and attractiveness of the odor, to determine the odor category (body, food, nature, etc.) and to freely describe the odor. The preliminary results of this study will be presented. Sex differences in detection and/or perceived quality are expected for the body-related compounds. Future data collection will involve younger and older participants, and women in various stages of the menstrual cycle, to test how modifications in reproductive ability and fertility might influence the perception of these compounds.

Identification and characterization of Drosophila food-derived pheromones influencing larval behavior

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Insects use chemosensory cues to feed and mate. In Drosophila, the effect of pheromones has been extensively investigated in adults, but rarely in larvae. Our data show that both larval and adult pheromones can specifically influence larval food search and the choice of a pupariation site. This indicates that these substances affect the dispersion and survival of Drosophila species in nature.

We recently showed that the colonization of natural food sources by *Drosophila buzzatii* and *D. simulans* species partly depends on species-specific chemical cues left in the food both by larvae and adults[1]. In these two species, we identified some of the pheromones and measured their influence on larval food preference and pupariation behavior[2]. We are now focusing our effort on *D.melanogaster* larval behavior and testing the behavioral effect of several larval-produced compounds. We have also started to map the neural pathway underlying their perception. We also compare the behavioral responses of transgenics (invalidated for cells involved in pheromonal perception) and several wild-type lines. We will present our most recent data obtained with these different species and populations.

Chemo- and thermosensory signaling in the Grueneberg ganglion

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The Grueneberg ganglion (GG) - a cluster of neurons in the anterior nasal region – is considered as an olfactory organ. We have recently identified odorants activating GG neurons, in particular given pyrazine analogues. Responsiveness to these compounds occurred in GG neurons characterized by the expression of the olfactory receptor V2r83, the guanylyl cyclase GC-G and the cyclic nucleotide-gated ion channel CNGA3. Experiments with knockout animals disclosed that GC-G and CNGA3 are important for odor-evoked GG responses.

GG neurons were also found to be activated by cool temperatures. Investigating the relevant signaling mechanisms revealed that almost all V2r83-/GC-G-/CNGA3-positive GG neurons responded to coolness, i.e, the same subset of GG neurons is activated by coolness and the above mentioned odorants. Searching for thermosensory proteins in the GG, the thermosensitive ion channel TREK-1 was found to be expressed in numerous GG neurons. Moreover, in TREK-1-deficient mice, GG responsiveness to coolness was reduced. However, even in the absence of TREK-1, GG neurons clearly responded to cool temperatures, suggesting that in addition to TREK-1, another thermosensor must be present in the GG. In this context, we observed that that the guanylyl cyclase GC-G is an unusual enzyme which is directly activated by cool temperatures. Consistently, in GC-G-deficient GG neurons, responsiveness to cool temperatures was largely reduced, indicating that this enzyme serves as a major thermosensor in cells of the GG.

Olfactory sensitivity in elderly with Parkinson's disease

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OBJECTIVES: Hyposmia is an interesting and a most common pathological characteristic of Parkinson's disease [Doty, 2013; Foguem and al, 2014] and is considered as a PD marker mainly at the early stage of the PD, in younger patients (< 65 years). Because few studies have considered olfactory loss in elderly patients with PD, the aim of this study was to assess olfactory function of elderly patients with idiopathic PD using odour detection thresholds compared to controls paired by age.

METHODS **:** Olfactory detection thresholds of Phenyl Ethyl Alcohol (PEA) [activating the olfactory system] and n-Butanol (BUT) [activating both olfactory and trigeminal systems], were determined in ninety-two patients with PD aged over 65 years old [mean age: 74.8 +/- 8.8 years, range: 65-93 years] and in ninety-two healthy controls matched for age and gender [mean age: 79.8 +/- 8.8 years, range: 65- 90 years]. The study also included neuropsychological evaluations and stage of PD estimations.

RESULTS: The results show significant impaired olfactory (CN I) detection sensitivity in relation to PEA thresholds in patients with PD compared to controls independently of age and stage of PD. There was also significant impaired detection BUT thresholds. The differential value between mean PEA dilution thresholds (controls – PD) was greater than the differential value between mean BUT dilution threshold (controls – PD). PEA and BUT thresholds were significantly correlated in both patients with PD and controls.

CONCLUSION : The findings of this study show that olfactory sensibility deficits mediated by PEA and BUT observed in young patients (<65 years) with PD are also observed in older patients (> 65 years) compared to controls paired in age.

Evolution of spatially coexpressed families of pheromone receptors in rodents

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In rodent VNO, pheromones are detected by two types of receptors namely V1Rs and V2Rs. Mouse V2Rs are expressed in the basal neurons and are organized in four families (A-D). Families-ABD V2Rs are expressed monogenically and coexpress with family-C V2Rs belonging to either subfamily C1 or subfamily C2, according to a coordinate diagram. Neurons expressing the phylogenetically ancient subfamily C1 coexpress families-BD V2Rs or a specific group of family-A V2Rs, whereas a second neuronal subset (subfamily-C2-positive) coexpresses a recently expanded group of family-A V2Rs (subfamilies A1-5) along with vomeronasal specific Major Histocompatibility Complex proteins, H2Mv. Here, we have analyzed the expansion of the V2R repertoire inferring the onset of the different families during the evolutionary history of Rodentia. Our results suggest that the separation of subfamily-C1 and -C2 V2Rs occurred in Cricetidae along with the origin of H2Mv molecules; however, this event did not correspond with the origin of subfamilies A1-5 which took place approximately 25 million years ago earlier in the Dipodidae lineage. The establishment of subfamily-C2 V2Rs that probably reflects the dramatic expansion of family A, generated receptors that have acquired a more subtle functional specificity.

Testing rat's neonatal response to flavors experienced prenatally

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Rat fetuses can perceive chemosensory stimuli derived from their mother's diet, and they are able to learn about these stimuli. Some studies in human and animals report that exposure to a flavor during gestation increases postnatal acceptance or preference of those flavors. In previous studies from our laboratory, we observed that prenatal exposure to ethanol during the last days of gestation (17-20) resulted in increased palatability and intake of ethanol in the infant rat. However these results were not found when rats were exposed during those same days to vanilla, cineole or anise. One hypothesis to explain these different results is based on the pharmacological properties of ethanol, which the other two stimuli lack of. However, considering that in those studies in which this effect was reported, subjects were tested before infancy; we explored the possibility of observing a differential response to flavors exposed those 4 prenatal days, when tested during the neonatal period using different techniques. In experiments 1 and 2, five-day old rats exposed to vanilla on gestational days 17-20 were tested with operant conditioning and intake tests. With the operant conditioning procedure, vanilla preexposed pups showed increased responses to vanilla as a reinforcer but not increased consumption of this flavor. In experiment 3, pups prenatally exposed to vanilla were tested with the "crawling" technique. Pups that were presented the same odor preexposed in utero crawled a greater distance towards the experienced flavor than control pups. In conclusion, our results indicate that prenatal exposure to a non-ethanol flavor induces increased acceptance but not intake of this flavor, although detectable only on neonatal stages.

Dietary bitterness reduces feed intake in piglets with or without gastrointestinal discomfort conditioning

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The bitter taste system in vertebrates mediates the onset of avoidance response to potential toxic plant compounds. Pigs have been shown to decrease their voracity when perceiving a bitter compound in the diet. For example, dietary addition with Bitrex resulted in a period of 3 to 5 days before pigs recovered their normal intake (Blair and Fitzsimons, 1970). The relatively short adaptation to bitterness may have been related to the lack of adverse health symptoms. The objective of this study was to assess the effect of gastrointestinal discomfort on the adaptation of piglets to a feed made bitter by using a non-toxic bitter compound. Seventy-two piglets (26.1 ± 2.4 days-old, initial BW 7.6 ± 0.6 kg) were selected at weaning, distributed into 24 pens (3 males or females per pen) and four experimental treatments (T1-T4), and were assigned following a randomized block design. In phase 1 (conditioning phase), piglets were meal fed twice daily at 8:00-9:00AM and 3:00-4:00PM two types of diet (control for T1 and T2 vs. bitter - quassia extract, 5 g/kg- for T3 and T4). The conditioning consisted of a 30' post-meal administration of 20 ml of tap water for piglets in T1, T2 and T3, and 20 ml of 3 LiCl solutions of increasing concentration (1.25%, 2.5% and 3.75% on Days 6, 7 and 8, respectively) for piglets in T4. After a 2-day recovery period, the experimental period (phase 2) started consisting of piglets fed ad libitum either the control (T1) or the bitter (T2, T3 and T4) diets. Thus, T2 was the bitter-naïve group in phase 2, whilst T3 and T4 had been exposed to bitter without or with discomfort respectively. During the first 2 days of feeding, feed intake in T2 pigs (unconditioned to bitter) and T4 pigs (LiCl-conditioned) tended (P <0.10) to be lower than in T1 piglets (control animals). On Day 3, T2, T3 and T4 treatments showed a lower (P <0.05) feed intake than T1. However, in Day 4, only T4 pigs maintained a lower (P =0.05) feed intake than control pigs. From Day 5 onwards no significant differences (P>0.10) were found between any of the treatments. These results show that dietary incorporation of a bitter compound results in a reduction in feed intake in piglets. The conditioning of bitter taste to gastrointestinal discomfort generated prolonged the feeding adaptation of weanling piglets in one additional day.

Vision Transcends Olfaction in Object Discrimination

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Two of the three 100-digit numbers below are identical and one is different from the other two. Can you tell which one is different?

6589507962789939724067227641260174163683704817656125765324779074497975772214636141366576682661743161
6589507962789939724067227641260174163683704817656125765324779074497975772214636141366576682661743162
6589507962789939724067227641260174163683704817656125765324779074497975772214636141366576682661743161

The middle number is greater than the other two by exactly 1. You used your visual system to discriminate two numbers that differ by 1 part in 10^{100}, or one part in a googol. Furthermore, you did it in less than one minute without even using colors. Since each digit can have 1 of 10 possible values and can be recognized as one of 10 possible shapes, the 100 digits represent 10^{100} possible objects that humans can discriminate.

The olfactory system is very inferior. Using a triad test of olfactory mixtures of 10-30 components analogous to the visual test used in the above example, Bushdid et al.[1] calculated that humans could discriminate 10^{12} different olfactory stimuli. It took several hours of testing of each subject to arrive at this figure. Thus, the visual system is at least 10^{88} times better than the olfactory system in discriminatory power. Odor pairs with more than 50% overlap were difficult to distinguish. On the contrary, visual objects with 99% overlap are easily distinguished.

Bushdid et al.[1] compared odor quality discrimination with color discrimination to argue for superiority of the olfactory system. However, that is unnecessarily restrictive because it eliminates the one aspect of the visual system—its spatial character—where it uniquely trumps the olfactory system. In the blink of any eye, we see an elaborate scene of texture, color and motion. With one sniff we get a disembodied odor without spatial detail.

When we see a skunk, we can instantly recognize it, determine its distance and direction of movement, and decide if it might create an unpleasant encounter. With smell alone, we have little spatial information and might be tricked by someone with an open vial of butyl mercaptan. We might not even smell the skunk at all, depending on the animal's disposition and the direction of the wind.

Imagine having to tell time, drive a car or catch a ball using only odor cues. If each letter of this abstract could be coded by an odor, reading would be very slow and tedious. Olfaction is very useful in discriminating volatile chemicals, but it cannot compete with the visual system in making fine distinctions among objects.

Inhibition of the mammary pheromone catabolism by a second odorant present in the same mixture

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The clearance of odorants from the perireceptor olfactory environment, involving odorant catabolizing enzymes, is increasingly believed to have a significant influence on olfactory sensitivity maintenance and therefore on perception. We have recently shown that the mammary pheromone (MP) is enzymatically conjugated to glutathione in rabbit olfactory epithelium (Legendre et al. 2014). This activity is particularly strong in newborns, i.e. in individuals behaviourally responsive to the pheromone, and higher in their olfactory epithelium compared to liver (although this latter is considered as the main xenobiotic metabolizing tissue). On the basis of these results, we hypothesized that a competitive enzymatic inhibition of the MP could occur when the MP is in mixture with a competitor odorant. Such competition in mixture between stimuli conjugated by the same enzymes has been widely described in drug metabolism, but never to date in olfaction. Here, a competitive effect on the MP glutathione conjugation induced by the presence of a second odorant in mixture with the MP was measured *in-vitro*(liquid chromatography), while the same competitive effect was measured *ex-vivo* on the global metabolism. The original *ex-vivo* method developed consists in measuring (headspace gas chromatography) the disappearance of an odorant (or a mixture) injected in a 20 ml sealed vial containing a fresh total explant of newborn rabbit olfactory epithelium. *In-vitro*, several odorant aldehydes were identified as strong inhibitors of the MP catabolism. This effect was confirmed *ex-vivo* attesting that glutathione conjugation is the major activity directed to the MP. Thus, our results presents the first demonstration that odorants in mixture can metabolically compete, leading to the inhibition of the catabolism of the odorant showing probably the less affinity for the enzyme. Such mechanism suggests that one of the odorant can accumulate in the perireceptor space and either activates more receptors or saturates them, and that its detection and perception is therefore modulates. We are currently investigating this competitive enzymatic effect on the *in-vivo* perception of the mammary pheromone.

Modulation of free-flight response in Drosophila.

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During insect flight, the fluid turbulence with the patchy and variable nature of odour plume itself impose a high degree of temporal fluctuation in the sensory exposure. To track an odour source, flies use across-antennae bilateral comparison of odour intensity to actively orient up a spatial odour gradient. The aim of our study consists to determine the contribution of wing sensory organs in odour-driven orientation behaviour. We used a wind tunnel to measure and compare the three-dimensional flight behaviour to an attractive odour in male and female flies of 6 Drosophila species. We detected important variation in several flight parameters according to the sex and species. To test the function of wing chemosensors in orientation behaviour, we targeted specifically in the wing margin the RNAi of the *Pox-neuro* (*poxn*) gene, which is involved in the determination of chemosensory organs. Such manipulated flies showed a decreased ability to initiate flight while their localization of the odour source remained unaffected. We also examined the effect of a food mixture containing acetoin. Our data suggest that this compound can modulate the time course to reach the odour source. In conclusion, our results indicate that flight trajectories in Drosophila are dependent of sex and species, while the wing sensors have a restricted role in flight initiation whereas acetoin can modulate flight speed.

Support: Gustaile ANR

Human event related potentials in response to oral stimulation with water

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How or even if the human brain processes the perception of the water in mouth is unclear. Some studies using functional magnetic resonance imaging, EEG-derived event-related potentials or magneto-encephalography show that water acts as a tastant (de Araujo 2003, Hashida 2005, Murayama 1996) producing activations in cortical taste areas like frontal operculum/anterior insula and/or the caudal orbitofrontal cortex. Other studies indicate that tastes to water arise after tastants from the mouth with a water rinse (Galino-Cuspinera, 2007). In contrast, others studies do not show a brain response to water (Kobajakawa 1996, Onoda 2005) but they suggest to use water as a tasteless solution for baseline.
Considering that in experiments that focus on gustatory perception the baseline (or the off condition) has a fundamental methodological significance, in our study we investigated the electrophysiological response of the human brain to water. To this end we used a computer-controlled gustometer which has been demonstrated to be able to elicit gustatory event-related potential (gERPs). The device is based on a pump system that allows to one deliver quasi-rectangular gustatory stimuli. Four conditions were chosen: 1. Somatosensory, 2. Temperature, 3. Taste, 4.Water. The conditions were presented on the tip of the tongue in a controlled experimental environment. Although EEG was recorded in 128 positions, here we discuss only a 5-channel subset, Cz, Pz, Fz, C3 and C4.
As a result we found a clear cerebral response under all 5 conditions, including water. The statistical analysis on the ERPs component indicated a significant difference ($p<.001$) between the conditions among latencies, P1, N1 and Plate, and among the peak-to-peak amplitude, A(P1-N1) and A(N1-Plate). A multi-comparison test revealed that there was no significant differences at P1 and N1 latency and for the amplitude A(N1-Plate) between the water and taste condition. These last results suggest a similarity in the human brain process of perception of taste and water.

Gustatory Evoked Cortical Activity in Humans in response to saccharose stimuli

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Introduction: Gustatory Evoked Potentials (GEP) can be detected in response to an intermittent stimulation of the gustatory receptors by a primary flavour. Contrary the other evoked cortical potentials (visual, auditory or sensory), GEP have not been extensively studied due to their recording heterogeneity. Our aim was to establish a reliable recording of GEP in Humans.

Methods: Voluntary and healthy subjects were included in this experiment. On the one hand, a saccharose solution with several concentrations (5, 10 or 20g per 100mL) was used as the gustatory stimulus, with an interval of at least 2 hours after eating or drinking. On the other hand, a 10g saccharose solution per 100mL of water was used before and after a standardized meal. Each session was performed on a different day. Control session was performed using water or no stimulus. The saccharin solution was presented to the median part of the tongue by intermittent stimulation. The intermittent stimuli were monitored by a specific equipment built for this purpose. Each 1s stimulus was presented 20 times with an inter-stimulus interval of 1min water. GEP were recorded from 9 cortical sites with EEG sensors: Cz, Fz, Pz, C3, C4, F3, F4, Fp1 and Fp2 of the 10/20 system (referenced against linked earlobes). GEP were obtained after average of all the responses.

Results: Thirteen healthy subjects participated in the experiment: 8 women and 5 men, from 25 to 34 years old (mean age 28). GEP consisted in one negative wave, without differences between men and women: their latency varied from 140 to 181ms and their amplitude fluctuated from 7 to 24µV. There is a good inter- and intra-individual reproducibility. None GEP was obtained during the control sessions. No difference was observed between the different saccharose concentrations. However, subjects with high saccharose liking had greater amplitude of the GEP than subjects with poor saccharose liking. Indeed, the latency of GEP increased after meal (11 healthy subjects, mean age 28 ans).

Conclusion: These data demonstrate that record GEP in Humans in response to saccharose stimuli is reliable. The differences in recording GEP (GEP amplitudes or latencies) reflect modification of cortical treatment in response to gustatory stimuli. This could be explained by cortical gustatory slowing after meal and increased gustatory signal with liking?

Short-term impact of a Western diet on the physiology of the peripheral olfactory system

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Current feeding behaviors contribute to the epidemic levels of obesity and diabetes observed in Europe and worldwide. Both the quantity and the quality of ingested food are incriminated. Together with other sensory modalities olfaction is involved in the control of food intake. However not only can olfactory cues influence eating behaviors, but nutritional status and diet can also alter olfactory abilities. Patients with metabolic disorders present impaired olfactory sensitivity which could in turn worsen their eating behaviors.

Here we examined the short-term impact of a Western diet high in fat and sugar (HFHS) on the anatomy and physiology of the olfactory epithelium of postnatal mice. We used a transgenic line of mice expressing GFP under the promoter of the SR1 odorant receptor in order to monitor the properties of a define population of neurons. After 8 weeks, HFHS animals presented a higher glycaemia, higher adiposity but no overweight compared to control mice. We measured electro-olfactogram amplitudes in response to two ligands of the SR1 olfactory receptor: amyl acetate and (R)-(+)-carvone. Detection thresholds of amyl acetate estimated from the dose-response curves were higher after 8 weeks of a HFHS diet (medians were 10^{-5} M for control *vs* 10^{-3} M for HFHS, $p < 0.01$). Reconstruction of the SR1 olfactory sensory neurons' cilia revealed shorter cilia in HFHS mice compared to control animals (4.5 ± 0.3 µm vs 6.0 ± 0.3 µm, $p < 0.01$).

Our results demonstrate that an enriched diet can alter the physiology of the olfactory epithelium on a very short term. Anatomical changes of individual ORNs may generate the reduced olfactory sensitivity. Olfactory dysfunctions appear early on after exposure to a Western diet.

Live imaging uncovers the mechanisms of taste sensory organ assembly

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Mature taste buds are composed of three major distinct cell types, named upon morphological criteria type I, II and III that functionally correspond to support, taste receptor and presynaptic cells. These three major cell subtypes are conserved in jawed vertebrates with some variability in their molecular content. Several elegant mammalian and amphibian studies have to contributed to our understanding of the mechanisms underlying the induction of the taste bud organs. A little less is known about the differentiation of these distinct cell types (1). We have shown that Fgf and Notch signaling regulate the expression of mir-200 that is required for taste receptor cell formation (2). Based on these results, we have constructed tools to observe the assembly of differentiating cells into a taste bud organ, in live zebrafish. I will present unpublished data showing how cells do differentiate within the oropharyngeal epithelium and assemble into a taste bud organ and discuss the underlying molecular mechanism of this process.

Impaired neurogenesis in the subventricular zone and olfactory functions by soft-diet feeding were recovered by hard-diet feeding

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Newly generated neurons at the subventricular zone (SVZ) migrate to the olfactory bulb (OB) and differentiate into granule cells and periglomerular cells. A reduction of mastication has been shown to impair both neurogenesis in the hippocampus and brain functions. To examine these effects on neurogenesis in the SVZ and olfactory function, we first showed that soft-diet feeding could mimic impaired mastication. BrdU-immunoreactivty of the SVZ and in the OB of mice fed a soft or hard diet were studied to explore the effects of changes in mastication on newly generated neurons. After 1 month, the density of BrdU-ir cells in the SVZ and OB was lower in the soft-diet-fed mice than in the hard-diet-fed mice. Avoidance, which were tested in a Y-maze apparatus, of butyric acid was reduced by the soft-diet feeding. We then explored the effects of the hard-diet feeding on olfactory functions and neurogenesis in the SVZ of mice impaired by soft-diet feeding. At 3 months of hard-diet feeding, avoidance of butyric acid was reversed and responses to odors and neurogenesis were recovered in the SVZ, suggesting that feeding with a hard diet improves neurogenesis in the SVZ and enhances olfactory functions at the OB.

Temporal dominance of sensations (TDS) analysis of binary taste mixtures

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Temporal dominance of sensations (TDS) is a sensory analysis tool to investigate time course of flavor perceptions during food consumption. The authors already reported about the TDS studies of several mono-tastant solutions, such as MSG(umami), lactic acid(sourness) and NaCl (saltiness) solutions. Each tastant solution showed increase in the duration time of corresponding taste modality in a concentration-dependent manner. In this study, we applied TDS to analyze binary taste-taste interactions such as umami-sourness, umami-saltiness, and saltiness-sourness, which may contribute to evoke complex gustatory sensations perceived in culinary broths. Three combinations of binary taste solutions (MSG and lactic acid, MSG and NaCl, and NaCl and lactic acid) are prepared at various levels of concentration and provided to trained panelists for TDS. The data were analyzed to obtain TDS curve, trajectory PCA of dominance rate, and duration time. Dominance rate and duration time of one basic taste modality are significantly affected by coexistent taste modality, and its details are dependent on the combination of modalities. This may suggest sensory competition of two taste modalities based on several factors occurs during taste mixture consumption.

Role of CD36 and GPR120 in fatty acid-mediated Ca2+ Signaling in human and mouse taste bud cells

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Oral perception of dietary fat was until recently thought to involve mainly texture and olfactory cues; however, accumulating evidence strongly suggests existence of a taste modality, devoted to the detection of long-chain fatty acids (LCFA). Mice can recognize dietary fat and FA solutions in the oral cavity in the absence of olfactory or textural cues. Hence, it is important to increase our understanding of gustatory detection of dietary fat and its contribution to fat preference. We studied the roles of the fat taste receptors CD36 and GPR120 and their interactions via Ca^{2+} signaling in fungiform taste bud cells (TBC).

We measured Ca^{2+} signaling in human TBC, transfected with small interfering RNAs (siRNAs) against mRNAs encoding CD36 and GPR120 (or control siRNAs). We also studied Ca^{2+}signaling in TBC from *CD36*$^{-/-}$ mice and from wild-type lean and obese mice. Additional studies were conducted with mouse enteroendocrine cell line STC-1 that express GPR120 and stably transfected with human *CD36*. We measured release of serotonin and GLP-1 from human and mice TBC in response to CD36 and GPR120 activation.

High concentrations of linoleic acid induced Ca^{2+} signaling via CD36 and GPR120 in human and mice TBC as well as in STC-1 cells, whereas low concentrations induced Ca^{2+} signaling via only CD36. Incubation of human and mice fungiform TBC with lineoleic acid downregulated CD36 and upregulated GPR120 in membrane lipid rafts. Obese mice had decreased spontaneous preference for fat. Fungiform TBC from obese mice had reduced Ca^{2+} and serotonin responses but increased release of Glp1, along with reduced levels of Cd36 and increased levels of Gpr120 in lipid rafts.

CD36 and GPR120 have non-overlapping roles in TBC signaling during oro-gustatory perception of dietary lipids; these are differentially regulated by obesity.

Genetic control of specific anosmia to androstenone in a mouse model

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Approximately 50% of adult humans have low olfactory sensitivity (specific anosmia or, more precisely, hyposmia) to the sex boar pheromone androstenone (Amoore, 1977; Labows & Wysocki, 1984). A functional role for this phenomenon in humans remains unclear, although it was suggested that androstenone is involved in human chemical communication. We have developed an animal model for specific anosmia to androstenone using inbred strains of mice CBA/J (CBA) and NZB/BlNJ (NZB). CBA mice detected androstenone at a concentration 2000-fold lower than did NZB mice (Voznessenskaya et al., 1995). We measured androstenone thresholds in F1 and F2 hybrids between the NZB and CBA strains using a training procedure with positive food reinforcement. The observed segregation of androstenone threshold phenotypes in F2 hybrids allowed us to perform chromosomal linkage analysis to map quantitative trait loci (QTLs) for androstenone sensitivity. DNA purified from tail biopsies of the F2 mice was used for a genome-wide scan with 98 microsatellite and 41 single nucleotide polymorphism (SNP) markers. An association analysis performed using R/QTL software (Broman et al, 2003) revealed suggestive QTLs for androstenone thresholds to mouse chromosomes 2, 5, 11, 12 and17 and a significant male sex-specific QTL to chromosome 10. Genes located within the QTL intervals were investigated to find candidates that either are olfactory receptor genes or are implicated in genetic control of olfactory sensitivity to androstenone in humans (Keller et al, 2007, Knaapila et al, 2012), and which are also polymorphic between the parental strains, CBA and NZB. We have found that the QTL on mouse chromosome 12 has conserved synteny with the region on human chromosome 7, which was previously associated with androstenone sensitivity in humans. Overall, our findings demonstrate value of using the mouse model to understand mechanisms of specific anosmia to androstenone. Funding Support: TW00495 NIH to VVV & CJW, NIH DC00298 to CJW, NIH DC00882 to AAB.

Dogs and mice detect odor signatures of hepatocellular carcinoma in urine of mice with experimental tumors

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Treatment success of cancer depends on its early detection. There are no biomarker identified to date in urine and blood with sensitivity and specificity sufficient for detecting cancer. However dogs and mice can smell early cancer signatures in urine and breathe samples. The biomarkers responsible for it could be volatile organic compounds. Animal cancer detection can be very useful for volatile biomarker discovery.

We use mouse cancer models to investigate dogs and mice ability to detect hepatocarcinoma. To create tumors, hepatocellular carcinoma H33 tissue was inoculated subcutaneously (n=79). For control purposes, 1 mL of saline was injected (n=91). Mouse urine was collected individually into a plastic tube. Urine samples were frozen at 20°C and retained until needed for experiments.

We use a match-to-sample-like protocol of scent identification lineups by dogs (n=4). The scent lineup consisted of 10 glass jars, each containing urine scent collected from a healthy mouse, and one jar with urine scent collected from a cancer mouse. A dog received the initial scent (collected from another cancer mouse) and was asked to match it to one in the lineup. We conducted three series of presentations to determine the urine samples from the mice after tumor inoculation, and one series in which one of the urine samples of the control group was collected from the same mouse whose urine was presented at the start, but before tumor inoculation. The accuracy of the dog's indication of 1-, 3- and 9-day tumor was 69.6%, 80.0%, 66.7% and sensitivity 95.2%, 97.3%, 94.7%, respectively.

We have conducted experiments with mice by modified "habituation-dishabituation" test. Each experiment included four presentations of odors. At the first, second and fourth presentation a sensor mouse sniffed the urine sample of the same healthy mouse donor after saline injection, and at the third, the urine sample of the same donor but 24 hours after tumor inoculation (n=20). In another experiment session, a sensor mouse sniffed the urine of an intact mouse against its urine after saline injection (n=16). The sensor mice reliably distinguished the urine of a healthy mouse from its urine after the hepatocellular carcinoma tissue inoculation, and also the urine of a mouse after saline injection from its urine prior to the damage.

Our studies showed that dogs and mice can have diagnostic success not only in detecting hepatocarcinoma signatures in urine, but also in discriminating between cancer and concomitant inflammation.

Inactivation of Gr5a-expressing taste neurons disrupts multiple taste modalities in the ingestion behavior of Drosophila

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The gustatory receptor (GR) gene, *Gr5a* , encodes a sugar receptor that are widely expressed in the gustatory receptor neurons of the labellum, legs, wings and other taste sensilla of the adult *Drosophila melanogaster* . In addition, there are some non-sugar *Gr5a* neurons that also innervate to a subset of the labellar taste sensilla, suggesting that some *Gr5a* neurons are multimodal. However, no functional studies analyzing the roles of those *Gr5a* neurons in the ingestion behavior have yet been carried out. In this study we analyzed the behavioral changes in the transformant strain, *Gr5a-GAL4>UAS-rpr* , where all *Gr5a* neurons are selectively inactivated by the exogenous expression of an apoptosis gene *reaper* (*rpr*) and compared the results with the control flies or with another transformant strain, *Gr66a-GAL4>UAS-rpr* , where all the bitter *Gr66a* neurons were instead inactivated, and evaluated the effects of dysfunction of *Gr5a* neurons on the proboscis extension, determination of intake volume, taste intensity discrimination , and the taste quality coding. As expected, the tarsal proboscis extension reflex by sucrose stimulation was severely impaired in *Gr5a-GAL4>UAS-rpr* flies. The total amounts of ingestion of sugar solutions in the flies, however, were not robustly affected while the discrimination between two different sugar concentrations was significantly reduced when the two sugar concentrations approach to equal. Interestingly, the preferential intake of sodium chloride solutions was also significantly disrupted in *Gr5a-GAL4>UAS-rpr* flies, suggesting that some Gr5a neurons are at least partially involved in the salty taste perception. Interestingly, we observed sexually dimorphic intake of salt solutions in drosophila, and *Gr5a-GAL4>UAS-rpr* flies exhibited weaker dimorphism than that of wild types. From the behavioral analysis we will discuss how each type of neurons contribute to the control of the feeding behavior.

Expression of ancestral V2Rs shifts from the main olfactory epithelium of tadpoles to the water nose of adult Xenopus laevis

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In mammals olfactory receptor families are segregated into different olfactory organs, chief among them the main olfactory epithelium (MOE) and the vomeronasal organ (VNO). In contrast, teleost fish olfactory receptor families are intermingled in a single sensory surface. To what extent such differences influence the coding and discrimination abilities of the respective olfactory systems is unclear, and the evolutionary path toward such segregation is unknown. Amphibians are early diverging tetrapods compared with mammals, and occupy an intermediate evolutionary stage concerning the water-to-land transition. Consequently, their analysis may shed light on this transition from shared sensory surface to segregated subsystems. We report here that a major olfactory receptor family of *Xenopus laevis* , V2Rs, is expressed in both olfactory organs of tadpoles, with the VNO expressing more 'modern', later diverging V2Rs, whereas more 'ancient', earlier diverging V2Rs are expressed in the MOE, together with the V1R family. Furthermore, amphibians such as *Xenopus* make their own ontogenetic transition from an obligate (tadpole) to a facultative aquatic stage in adults. During metamorphosis the MOE of *Xenopus* tadpoles transforms into an air-filled cavity (principal cavity, air nose), whereas a newly formed cavity (middle cavity) takes over the function of a water nose. We report here that larval expression of 'ancient' V2Rs is gradually lost from the main olfactory epithelium as it transforms into the air nose. Concomitantly, 'ancient' V2R expression begins to appear in the newly forming water nose. Responses to amino acid odorants are present in the tadpole MOE, and show the same transition, disappearing in the transforming air nose and concomitantly appearing in the new water nose, consistent with the hypothesis that amino acid responses may be carried by V2R receptors. Interestingly, 'ancient' v2r genes are expressed in a basal expression zone both in tadpoles and adults, so this feature of V2R expression is stable during the migration of expression from one olfactory epithelium to another during metamorphosis.

Age-related effect on gustatory event-related potentials and their brain sources

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Gustatory event related potentials (gERP) were first established by Funakoshi und Kawamura in 1971. In 1985 Kobal developed a device able to elicit gERPs which was in modified versions also applied by other groups (Hummel et al., 2010. Mizoguchi et al. 2002).
Aim of the present study was to use psychophysics and gERPs to investigate age-related differences in the processing of gustatory information as indicated by the cerebral sources of the gERP. A total of 96 subjects participated in the study. After olfactory and gustatory screening for normal function the volunteers were invited to two sessions of gERP acquisition. Subjects received a randomized combination of five iso-intense basic tastants presented at a medium-level . To record gERPs a 128-channel EEG system (BioSemi, Amsterdam, NL) was used. The whole group of participants was subdivided in four age sub-groups.
Psychophysical testing for smell and taste function included the “Sniffin’ Sticks” (Hummel et al. 2007; Kobal et al. 1996) and the “Taste Strips” (Landis et al. 2009; Mueller et al. 2003). Both, olfactory and gustatory function exhibited a significant decrease with age. Currently analyses are underway to investigate age-related changes in electrical brain activity and their sources

Evidence of interactions between aroma compounds and the CB1 receptor opens new routes for regulation of food intake

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Obesity is a major cause of morbidity and mortality worldwide, characterised by a chronic imbalance of energy homeostasis. Reduction in dietary intake could be an effective way to counteract this imbalance. Among mechanisms involved in the regulation of food intake, it is well established that the endocannabinoid system influences appetite via the cannabinoid receptor type 1 (CB1). Agonists of CB1 can promote food intake while inverse agonists tend to decrease appetite. Interestingly, recent studies suggest that aroma compounds could have an impact on food intake through interactions with the endocannabinoid system.
To explore this hypothesis, we performed two computational studies to identify aroma compounds that could interact with the CB1 receptor:

- we investigated all biological targets potentially recognized by aroma compounds by navigating into the large chemogenomic database ChemProt. Based on protein identifiers (Uniprot), we identified 26 protein families as potential targets. Not surprisingly, the G-protein coupled receptor family (GPCR), which include CB1, is the most common type predicted.
- we generated pharmacophore models derived from chemical structures of known agonists and antagonists of CB1. Then we used these models to perform an *in silico* screening of around 3000 aroma compounds described in the Flavor-Base (Leffingwell and Assoc.).

After comparing the odorant molecules predicted as CB1 ligands by both approaches, nine molecules were selected and their capacity to activate the CB1 receptor was assessed in an*in vitro* functional assay using HEK293 cells expressing the mouse CB1 receptor. Interactions between candidate molecules and the CB1 receptor were determined by measuring cAMP production. Three molecules were found to be moderate inverse agonists of the CB1 receptor. In addition, measurement of CB1 mRNA in mouse tissues by quantitative real-time RT-PCR showed significant expression in the olfactory bulb and the olfactory mucosa.
On the basis of these findings, interactions between the endocannabinoid system and aroma compounds could be proposed as a mechanism involved in the establishment of satiety.

The olfactory context affects facial expression processing : An ERP study

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In a previous experiment, we found that olfaction influences the incidental processing of facial expressions in two ways. First, it enhances the event-related potentials (ERPs) in response to facial expression around 150 ms after stimulus-onset regardless of the emotional content of expressions and the hedonic value of the odorant. Second, it modulates the Early Posterior Negativity (EPN, approximately 220 ms after stimulus-onset) by increasing/decreasing the differential response to the various expressions.
Here, we further precise the effects of the olfactory context during facial expression processing by manipulating the intensity of the expressions. Adult participants (N=20) were required to tell whether faces were expressive or not and 3 types of faces were used depending on their expressiveness (neutral, ambiguous, expressive). Ambiguous and non-ambiguous expressions were created with a morphing software and were selected so that ambiguous faces were rated "expressive" in 50% of responses during a pre-test. Participants performed several blocks in 3 olfactory contexts varying from one block to the other: control (scentless air), pleasant (strawberry) and negative (butyric acid).
The results showed that the intensity of facial expressions enhanced ERPs as soon as 40 ms after stimulus-onset and lasted over a period including the P100 (100 ms) and N1/N170 (150 ms) components at occipito-parieto-central sites, indicating an *early main effect of intensity of expression* irrespective of the emotional content of faces. At occipital sites, this effect corresponded to a linear increase from neutral to non-ambiguous expressions whereas it differentiated non-ambiguous expressions from both neutral and ambiguous expressions at parietal sites. The *first differential effect of expression* appeared at occipital positions at the level of the EPN (around 220 ms). Importantly, the olfactory context was influential on the visual processing of emotional faces in two ways: 1/ a global effect emerging around 150 ms after stimulus-onset whatever the emotional content of expressions. 2/ an interaction between odor, the intensity and the content of expressions occurring in the EPN around 220 ms. These results suggest that the olfactory context has an undifferentiated influence on the early perceptual categorization of expressive faces (150 ms) before its hedonic value interacts with the processing of the affective content of expressions depending on their intensity (220 ms). They are discussed in the light of current theories of multisensory integration of vision and olfaction.

Chemical and Behavioral Approaches in the Elucidation of Olfactory Interactions Between Human mothers and newborns

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Odor stimuli and their conveyed information have been investigated in mother-infant dyads of several mammalian species. Correlational and experimental studies have shown that maternal milk and breast odor influence the behavior of human neonates, e.g. in modulating arousal or enhancing the duration of rooting and oral movements in a breastfeeding-like situation. However, the chemical bases explaining the described effects are unknown. Here, we present a bunch of analytical and ethological studies exploring the behavioral impact of biological odorants in the perinatal environment.

Gas chromatography-olfactometry and ultra-performance liquid chromatography-mass spectrometry were applied to study odorous compounds and non-odorous precursors thereof in amniotic fluid and human milk. Amongst others, odorous steroids and amino acid-conjugates of sulfanylhexanols and branched acids known to be present in sweat were detected and quantified in these fluids. Guided by these results, the respective odorous compounds were orthonasally administered to 3-days-old newborns in episodes of active sleep. The respiratory rate and facial activity of the neonates were recorded to infer olfactory detection and hedonic value of the odorants. No stimulus-specific respiratory response was evident. Further, whereas odorous steroids elicited a relative increase of negative facial actions, only marginal behavioral effects were observed for the other odorants. These results demonstrate perinatal chemosensory continuity on a molecular level and suggest that part of human amniotic fluid/ milk odorants is of negative valence for neonates, at least in the present experimental conditions when they are presented in pure form and high dilutions.

Expression level of nAChRs in taste buds influence the Neural and Behavioral Responses to Nicotine and Ethanol in Alcohol Preferring (P) and alcohol Non-preferring (NP) Rats

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Taste responses to nicotine and ethanol involve both TRPM5-dependent and TRPM5-independent transduction pathways (Oliveira-Maia et al. 2009). The TRPM5-independent nicotine and alcohol responses depend upon the presence of nicotinic acetylcholine receptors (nAChRs) in taste cells. We investigated if differences in behavioral and neural responses to nicotine and ethanol between alcohol preferring (P) and alcohol nonpreferring (NP) rats are related to the differential expression of nicotinic acetylcholine receptors (nAChRs) in taste bud cells. Naïve P rats demonstrate Preference for 5% ethanol and 10 μg/ml nicotine relative to naïve NP rats. P and NP rats responded with a dose-dependent increase in chorda tympani (CT) taste nerve response to increasing ethanol and (-) nicotine free base (NFB) concentrations. The maximum normalized tonic CT response to ethanol in P rats was higher than in NP rats. In P rats the relationship between NFB concentration and the normalized tonic CT response was shifted to lower concentrations relative to NP rats. CT responses were monitored in P rats while stimulating their tongue with varying ethanol concentrations (5-40%) at a fixed nicotine concentration (5 mM) or with varying nicotine concentrations (1-20 mM) at a fixed ethanol concentration (30%). Increasing ethanol concentration at 5 mM nicotine or increasing nicotine concentration at 30% ethanol, produced biphasic effects on the CT response relative to the H_2O rinse. At 5 mM nicotine, ethanol increased the CT response at 20% and 30% and inhibited the response at 40%. AT 30% ethanol, the CT response reached the peak at 2.5 and 5 mM nicotine then decreased at 10 mM. Real time PCR data showed that the mRNA levels for α4, α5, α6 and β4 nAChR subunits in circumvallate (CV) taste buds of naïve P rats were higher than naïve NP rats. Rats were treated with 5% ethanol for 3 weeks in a free choice paradigm. Following ethanol exposure P rats demonstrated increased mRNA expression of α5 and α6 and NP rats showed an increase in α6 and β4 nAChR subunits. Similar to NP rats, TRPM5 knockout mice also showed an increase in β4 nAChR subunit upon exposure to 5% ethanol. We conclude that the nAChR expression levels in taste cells correlate with neural and behavioral responses in naïve and ethanol treated P and NP rats through a TRPM5-independent pathway.

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Expression of c-Fos in the anterior piriform cortex after acquisition of conditioned flavor preference

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Taste aversion learning (TAL) and conditioned flavor preference (CFP) facilitate animal survival and play a major role in food selection, but the neurobiological mechanisms involved are not completely known. The present study propose to examine the neuroanatomical bases of CFP using recording neuronal activity by immunohistochemistry for Fos. Three groups (group 1, 2, and 3) of male rats were trained over 8 alternating one-bottle sessions to acquire a CFP *flavor-flavor* induced by pairing a flavor (grape/cherry) with saccharin. In an additional control group (group 4), the same flavors were presented in water without saccharin. The day immediately following training, the animals of groups 1, 2 and 4 were offered the grape flavor (7 ml) in a 15 min session. Two hours after drinking session, the rats were deeply anesthetized and brains were imunohistochemically processed for c-Fos. Two choice-test were given to group 3 and behavioural data showed that the animals in this group were capable of learning the flavor-saccharin association under these experimental conditions. Neurons showing Fos-like immunoreactivity (FLI) were counted in infralimbic cortex (IL), accumbens nucleus, *core* (AcbC) and anterior piriform cortex (aPC). Immunohistological analysis showed a significant higher number of activated cells only in piriform cortex in groups 1 and 2 *vs* group 4. Results obtained appear to indicate that the learning process might have produced a plastic change in the aPC. Further studies are required to determine the functional relevance of piriform cortex in the flavour-taste learning.

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PROP bitter taste-induced brain activation-deactivation in gustatory signal processing: a functional magnetic resonance imaging (fMRI) study

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Although human brain pathways involved in the complex gustatory circuitry are well known, there is still the need to elucidate how taste signals are processed in the brain and whether stimuli may influence, in the neural processing, different brain areas depending on the meaning that they have for individuals. Since many bitter substances are toxic, the genetic ability to taste the bitter quality confers an important survival advantage by allowing super-taster individuals to recognize potentially harmful substances which instead are not detected by non-tasters. In this preliminary fMRI (GE-SIGNA 1.5 Tesla) study we investigated the neural activity induced by the bitter taste of propylthiouracil (PROP) in six subjects (1 males, 5 females, age 28.6 ± 0.86 y) previously genotyped for receptor *TAS2R38* and classified for their PROP taster status. PROP was delivered to the subjects by filter paper disks impregnated with the compound. The four major regions of interest (ROIs) constituting the default mode network (DMN), e.g. the posterior cingulate cortex (PCC, the medial prefrontal cortex (MPFC), and left and right lateral parietal cortices (LLP and RLP), were examined as sources for the connectivity analysis. Targets were the complete set of Broadmann areas.

Three subjects were classified as PROP super-tasters and had a homozygous genotype for the taster receptor variant (PAV/PAV), while the other three were classified as non-tasters and had homozygous genotype for the non-taster receptor variant (PAV/PAV).

The activation of LLP source was inversely correlated with Premotor Cortex (left), Dorsolateral Prefrontal Cortex (right) and Retrosplenial Cingulate Cortex (right), and directly with Primary Visual Cortex V1 (left); the MPFC source was inversely correlated with Somatosensory Associative Cortex (right) and Anterior Entorhinal Cortex (right); the PCC source was directly correlated Anterior Cingulate Cortex (right), Anterior Cingulate Cortex (left), Subcentral Area (right), Somatosensory Associative Cortex (left) and Dorsal Anterior Cingulate Cortex (right); RLP source was directly correlated with Auditory Cortex (right) and Insula Cortex (left).

In conclusion, our results show the Cortical Areas in which PROP bitter taste signals are differently processed in two genetically different groups (PROP super-tasters and non-taster) consistently with individual differences in the ability to taste this compound.

Odors as an efficient tool in emotion regulation in humans

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A growing literature documents that emotion regulation may decrease negative affects. Automatic regulation processes have been identified, involving extrinsic factors due to the environment (e.g. music). Although the effects of odors on affects have already been described, there is no data in the literature about the potential of odors to regulate negative emotions induced in experimental situations.

The present study aims to examine the effect of pleasant odors on emotions induced by unpleasant pictures. The study also compares young and older subjects, as emotion regulation is usually claimed as more effective in older subjects.

24 younger and 12 older (60-76 years) subjects were tested individually in a quiet room. They saw 24 pictures selected from the International Affective Pictures System, either with a neutral or negative hedonic valence. Each picture was showed for 5 seconds in a random order. After each picture, a screen with a cross appeared for 8 seconds and a pleasant odor, either lavender or rose, was smelled by subjects for half of the pictures. The delivered flow of air was odourless for the other half. An olfactometer with a tubing output placed under the subject's nose was used for this purpose. Then the subjects had to rate their feelings induced by the previous picture on a Likert scale (from neutral to pleasant). Heart rate and electrodermal activity was continuously monitored during the experiment.

We hypothesise that negative feelings induced by unpleasant pictures decrease when followed by a pleasant odor compared to the odourless condition. We expected that this decrease would be greater for older subjects than for young subjects according to an age-related positivity effect.

The results (ANOVA) show that pleasant odors significantly decrease negative feelings induced by pictures $p<0.006$, either with a negative $F(1, 34) = 7, 24$; $p<0.01$ or neutral $F(1,34) = 4.57$; $p<0.04$ valence. Heart rate decreases during the odor condition compared to the odourless condition $F(1, 34) =9.25$; $p<0.005$. However, comparison of the ratings by young and older subjects failed to reveal any statistical difference.

Thus odors can enhance positive affect not only in a neutral emotional condition but also in a negative emotional situation and thus play a role in regulation processes. An age-related positivity effect was not observed, perhaps due to the specificity of the direct limbic neuronal pathways of this sensorial cue.

Structural, Functional and Epigenetic Responses to Olfactory Fear Extinction Training in Mice

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Olfactory sensory neurons (OSNs) of the Main Olfactory Epithelium (MOE) provide a rich model to study the perception of external cues and the underlying mechanisms regulating structural plasticity within the olfactory system. Using the M71-LacZ mouse line (OSNs expressing the M71 odorant receptor can be visualized by LacZ immunohistochemistry (Vassali et al., 2002)), we have demonstrated an increased number of M71+ OSNs in the olfactory epithelium following cue-specific olfactory fear conditioning to acetophenone (Jones et al., 2008), an odorant shown to specifically activate the M71 receptor. Furthermore, this increase in M71+ OSNs was directly correlated with an increase in the M71+ glomerular cross-sectional area and volume within the olfactory bulbs. Notably, when animals receive the same odor-shock pairing to another odorant that does not activate M71, there are no detectable changes in the M71 neuron population or glomeruli. Functionally, mice exhibit enhanced freezing to the conditioned odor stimulus following olfactory fear conditioning. These previously published data indicate that the olfactory nervous system responds both structurally and functionally to olfactory fear, however, it is unknown whether previously acquired responses to the conditioned cue can be reversed by cue-specific fear extinction. We sought to determine whether the behavioral (increased freezing) and structural (increased number of M71+ OSNs and M71+ glomerular area) changes observed after olfactory fear conditioning may be reversed with extinction training. Additionally, using native chromatin immunoprecipitation (N-ChIP) protocols on the MOE, we investigate the dynamic alterations in permissive and repressive histone marks around the M71 gene locus following both olfactory fear acquisition and extinction. Male mice were trained to associate mild footshocks with acetophenone using a session consisting of 5 odor-shock pairings (1 session/day for 3 days). 3 weeks after the last conditioning session, animals were handled only or exposed to an extinction session that involved the presentation of 30 acetophenone-only presentations (1 session/day for 3 days). 3 weeks after the last extinction session, animals were sacrificed. We demonstrate that extinction training specific to the conditioned odorant cue reverses the conditioning–associated increases in freezing and M71-specific OSN number and glomerular area. Furthermore, we demonstrate a dynamic regulation of histone marks around the M71 locus associated with both cue-specific fear learning acquisition and extinction. Our observations shed light on how the olfactory sensory system responds dynamically to extinction learning after fear conditioning.

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Odorant biotransformation by human gluthatione-S-transferases

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The human olfactory epithelium is continuously exposed to a large variety of chemicals including odorant molecules. In this tissue, catabolizing enzymes are involved in the biotransformation of these molecules facilitating their clearance as metabolites. Theses enzymes and other proteins including odorant-binding proteins participate to a series of processes taking in charge, in different manner, odorants of the peri-receptor environment and therefore likely contribute to olfactory perception. Some of these catabolizing enzymes have been already suggested to be involved in human olfactory perception. Olfactory glutathione-S-tranferases (GSTs) have been involved in olfaction in different animal species including drosophila or rabbit. GSTs are well known for their role in the conjugation phase (phase II) of the metabolism of xenobiotics. In humans, two GST have been identified in the olfactory mucus epithelium in a proteomic study: GSTA1 and GSTP1. Based on these results, suspecting a possible role of the GSTs in human olfaction, we explored the ability of these enzymes to glutathione conjugate odorants using a biochemical approach. We heterologously overexpressed GSTA1 and GSTP1 in *E. coli* . The proteins were purified at a high level of purity. Enzymatic tests demonstrated that these both enzymes catalyze specific odorant glutathione conjugation. The large quantity of pure enzyme allowed us to develop an odorant screening assay for 93 odorants belonging to various chemical classes. This binding profile established for each of the two GSTs demonstrated i) the ability of these enzymes to directly interact with a large panel of odorants, in absence of any other enzymes, and ii) the presence of a complementary and overlapping catalytic spectrum for these enzymes. We further investigated the affinity range for the different odorants, we observed, in odorant interacting experiments, that the molecules can be divided in different categories depending of their affinity ranges, showing specific properties of these enzymes towards odorants. By combining different biophysical approaches like thermal shift assay, fluorescence, and circular dichroïsm, we were able to determine the affinity constants (Kd) for some odorants. In addition, we found that the nature of the interaction of the odorant with the enzyme was specific: some odorants behaving as substrate others behaved as inhibitors. Our results give new insights concerning the peri-receptor events in olfactory process and the possible role of GSTs in human chemical senses.

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A Proximity Model for Synapse Formation of Mitral Cells in the Olfactory Bulb

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In the mouse olfactory system, map topography is largely established by axon-axon interactions of olfactory sensory neurons (OSNs). However, to make the map functional, the OSNs must make proper connections to second-order neurons; the mitral/tufted (M/T) cells. Then, how do the M/T cell dendrites find their partner glomeruli for synapse formation with OSN axons? Does the odorant receptor (OR) specificity of glomeruli play an instructive role in matching M/T cell dendrites with OSN axons? To address these questions, we analyzed dendrite selection and synapse formation of mitral cells in various mutant mice in which glomerular formation was perturbed. We also studied synapse reconstitution in adult glomeruli. Our present results support the "proximity model", whereby mitral cells tend to connect primary dendrites to the nearest neighboring glomeruli regardless of OR specificity. The map location or address code of glomeruli rather than the OR specificity plays a key role in matching mitral cells with their partner glomeruli. In this regard, synapse formation in the mouse olfactory system is different from those found in the fly and nematode where the olfactory circuit is genetically programmed and matching is pre-determined by cell lineage.

Olfactory Performance Is Predicted by Individual Sex-Atypicality, but Not Sexual Orientation

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Many previous studies have reported robust sex differences in olfactory perception. However, both men and women can be expected to vary in the degree to which they exhibit olfactory performance considered typical of their own or the opposite sex. Sex-atypicality is often described in terms of childhood gender nonconformity, which, however, is not a perfect correlate of non-heterosexual orientation. Here we explored intrasexual variability in psychophysical olfactory performance in a sample of 156 individuals (83 non-heterosexual) and found the lowest odor identification scores in heterosexual men. However, when childhood gender nonconformity was entered in the model along with sexual orientation, better odor identification scores were exhibited by gender-nonconforming men, and greater olfactory sensitivity by gender-conforming women, irrespective of their sexual orientation. Thus, sex-atypicality, but not sexual orientation predicts olfactory performance, and we propose that this might not be limited to olfaction, but represent a more general phenomenon.

The Sniffing Brain

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Sniffing is as an active sensing mechanism. Sniffing not only transports stimuli from the environment, but also drives multi-modal neuronal activity. Phase-locking of active sensing mechanisms with the respiratory cycle is widespread across the animal kingdom; from rodents synchronizing bouts of sniffing and whisking to moths and bats phase-locking neural activation with the incoming airflow created by their wing beat. Previous studies have demonstrated attentional effects of stimulus presentation on human respiration. Nevertheless, whether humans exhibit such respiratory-driven modulation of sensing or mentation remains unknown. With this in mind, we set out to ask whether respiration mode (nasal exhale/inhale) affects performance of simultaneously conducted cognitive tasks. First, we asked whether humans implicitly prefer to engage in cognitive tasks during a specific respiratory phase (inhale/exhale). To test this, we monitored nasal respiration of subjects as they engaged in spatial (n=29), mathematical (n=24), and verbal (n=26) tasks. Subjects self-initiated trial onset, and were instructed to answer as fast and accurately as possible. Each trial concluded in an inter-stimulus interval (5 – 15 s, randomized). Analysis of the respiratory phase at the moment of trial onset ("requesting a task") and trial end ("answering the task") revealed a strong preference for requesting trials during exhalation across all tasks (t -tests: spatial: $t(28)=3.14$, $p=0.004$, math: $t(23)=1.16$, $p=0.007$, verbal: $t(25)=5.48$, $p<0.0001$) as well as when submitting an answer in verbal and mathematical tasks (t -tests: math: $t(23)=2.29$, $p=0.032$, verbal: $t(25)=2.73$, $p=0.011$). In other words, humans implicitly choose to conduct tasks within a particular nasal respiratory phase. We are following up on this behavioral finding using fMRI to ask whether patterns of brain processing fluctuate accordingly as a function of respiratory phase. These findings imply two modes of brain activity, one associated with stimulus acquisition and one with stimulus processing. This view implies a novel role for olfaction in the evolution of all sensory and cognitive brain processes.

Density of taste receptor cell types in mouse circumvallate taste buds

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Taste responses are different among fungiform, circumvallate and soft palate taste buds. We compared the cell density of each taste receptor cell among these taste buds. Mouse taste receptor cells in peeled lingual and soft palate epithelia were classified with antibodies against PLCβ2 (a phospholipase), Gγ13 (a G-protein γ subunit), and SNAP-25 (a SNARE protein). We optically sliced taste buds from taste pore to the bottom of taste buds with a confocal laser-scanning microscope to count whole cells rather than conventional slicing, and investigated the number of each cell type and the horizontal cross section of taste buds. The maximal cross-section of circumvallate taste buds was 570±180 μm^2 (n=110), which was significantly smaller in fungiform and soft palate taste buds. The vertical length was significantly smaller in circumvallate taste buds than in the others. The number of total cells and that of each cell type linearly increased as a function of the maximal taste buds area. The density of total cells and that of SNAP-25-immunoreactive was significantly larger in circumvallate taste buds than in the others. The density of PLCβ2- and Gγ13-immunoreactive cells was significantly higher in circumvallate taste buds than that in fungiform ones. The density of PLCβ2-immunoreactive cells was similar in circumvallate and soft palate taste buds. These results showed that the density of each cell type depends on the region of taste buds, though TBCs were more tightly packed in circumvallate taste buds, and suggest that these differences affect respective taste nerve responses.

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Crosstalk Between Hypothalamic Neurons and Olfactory System: a Tracing and Anatomo-Fuctional Investigation in Mouse

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Food intake is controlled by complex neural systems involved in homeostatic function but also in information processing regarding previous experience with food and reward. This hedonic component elicited by palatable food sometimes overrides the homeostatic regulation. For instance, the smell of sweet food can triggers ingestion even in a satiated state. Such compulsory behavior likely contributes to the obesity epidemic. The lateral hypothalamus (LH) which is a central node in the brain feeding network receives olfactory inputs. Conversely, the olfactory bulb (OB), first cerebral relay of the olfactory system, is the target of hypothalamic efferent projection. In this context, orexins (ORX) neurons located in the LH, known to promote food intake through modulation of brain reward circuit, might have a pivotal role in the crosstalk between the hypothalamus and the OB. However, direct projection from the ORX neurons to the OB has not been evidenced (in mouse) yet. The first part of the study aimed at probing the hypothalamo-olfactory ORX pathway. Secondly, we investigated whether this network could integrate odors stimuli from palatable food. Using stereotactic injections of DiI in the OB combined with ORX immunodetection, we observed retrogradely labeled cells, which were also stained for ORX in LH. These results strongly suggest that ORX might be involved in modulation of olfactory reactivity according to food properties. Second, we examined the activation of ORX neurons in an olfactory-based hedonic feeding paradigm. In contrast to controls, sated mice had access for 15 min/d for 3 days to an appetitive sweet cereal characterized by a chocolate odor for 3 days. For the test, all mice were stimulated by the chocolate odor. To assess the neuronal activation, double immunocytochemistry of the immediate early gene c-fos protein product (Fos) and ORX was performed. Fos expression was significantly increased in LH, indicating the activation of this brain area by the food-derived odor. We observed that some ORX-labeled cells were Fos positive but no difference was noted between the two groups of mice. Additional experiments showed no co-localization of Fos with MCH (melanin concentrating hormone; another LH orexinergic peptide). Finally, our study demonstrates a direct projection from ORX neurons from LH onto OB. Neuronal activation of LH by an appetitive food odor was evidenced but the identification of the neurons remains to be performed.

Expression of butyrate receptor GPR43 in rats colon and the dietary fiber from blackberry

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Introduction: Short chain fatty acids (SCFA) like, propionate and butyrate are the major molecules produced by the bacterial fermentation of dietary fiber (DF) in the colon. Recently, the butyrate has been recently studied because is important to maintain colonic functions phisological and because it has been related with a protective effect in colorectal cancer, which is mainly, explained by its potential to regulate gene expression by inhibiting enzyme histonedeacetylase (HDAC). Several researches shown that SCFA receptor GPR43 is involved insignal transduction mechanisms once they bind to ligands such as propionate and butyrate to generate different physiological effects in the colonocytes the mammals.
Objective: Determine if dietary fiber consumption from blackberry *(Rubus fructocousus var. brasso)* containing a ratio of soluble-insoluble fiber 40/60, has a direct influence on the quantitative expression of butyrate-specific receptor GPR43.
Methods: Wistar rats were fed with four different diets formulated at different concentrations of dietary fiber of 0, 5, 10 and 15% of dietary fiber from blackberry, respectively and the expression was determined with rt-pcr quantitative.
Results and discussion: The results shown an increase in the expression of GPR43 (93.1%) when rats was fed with a 5% fiber diet, using β-actin as a reference gene. The results of this research will contribute to determinate the relation of diet (dietary fiber) with intestinal health for the purpose of expanding the knowledge of butyric acid on colonic functions physiological, mediated induction of the GPR-43 receptor as intestinal chemoreceptors.

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A Novel Semi Intact in Situ Nose-Olfactory Bulb-Brainstem Preparation (NOBBP) of Rat

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In this study we adapted an established technique, the semi intact *in situ* working heart brainstem preparation, to develop a NOBBP with preserved anatomy of the olfactory system from the nasal cavities until the olfactory bulb and preserved respiratory circuitry. In brief, rats were deeply anesthetized and after precollicular decerebration the preparation was perfused with a blood-like solution via the descending aorta. While the brainstem (BS) is oxygenated via the basilar artery, the olfactory bulb (OB) is perfused in parallel via the ophthalmic artery despite complete removal of the forebrain. Nevertheless, the anatomical integrity of olfactory nerves innervating the OB and nasal trigeminal afferents innervating the BS still allow for studies of odor processing within the OB and the respiratory BS *in situ* . Moreover, this preparation allows to study rhythmic network activity in the bulb during respiration and odor processing.

Recording of OB field potentials revealed spontaneous activity, particularly in the granular cell layer. Application of odorants (essential oils of menthol, lavender, peppermint, vanilla and rose) by venting the nasal mucosa using an animal ventilator (rhythmic airflow) or a computer controlled olfactometer (constant airflow during stimulation) reliably produced evoked olfactory field potentials and also oscillatory activity after stimulation. Parallel recordings from the phrenic nerve showed, that odors triggered significant and specific respiratory modulations via the trigeminal pathway. Room air stimuli of the nose caused weaker activation of the olfactory bulb than odorant exposure. Moreover, irrigation of the nose with cold water did not evoke field potentials but triggered a breath hold signal due to the protective diving reflex. A first set of single and multi-unit recordings revealed stimulus-selectiveness across different odorants with different amplitude responses.

We conclude that the NOBBP is an exciting new tool that allows for studies of primary odor processing in the olfactory bulb and in brainstem respiratory circuits in parallel, from the molecular to the systems level, while maintaining *in vivo-like* neural network function.

Calcium-Activated Chloride Channels in Isolated Mouse Vomeronasal Sensory Neurons

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The main function of Vomeronasal Organ (VNO) is the detection of pheromones controlling a large variety of physiological functions. The neuroepithelium of VNO is composed by various types of vomeronasal sensory neurons (VSNs) expressing different types of chemosensory receptors and by supporting cells. The sensory transduction process occurs in the microvilli, which are present in the apical region of the dendrite of VSNs. Although some steps of the transduction cascade are known, the complete signaling pathway remains elusive. The transduction process starts with the binding of molecules with vomeronasal receptors, leading to the activation of a G-protein and the subsequent activation of a phospholipase C signaling cascade, which activates a transient receptor potential canonical 2 (TRPC2) that allows Na^+ and Ca^{2+} influx. Ca^{2+}-activated Chloride Channels (CaCCs) are present in the apical region of VSNs. Moreover, immunohistochemistry showed the expression of the CaCCs TMEM16A and TMEM16B in the microvilli of VSNs. The aim of this study is to characterize the currents activated by Ca^{2+} in isolated mouse VSNs using the patch-clamp technique in the whole-cell configuration, dialyzing the neurons with different free Ca^{2+} concentrations. We recorded currents activated by submicromolar Ca^{2+} concentrations, reaching an average value of 1.5 nA at +100 mV with 1.5 mM intracellular Ca^{2+}. The current showed both a Ca^{2+} and voltage dependence. The dose-response for Ca^{2+} showed that the $K_{1/2}$ decreased at higher membrane voltage ranging about 1.4 mM Ca^{2+} at -100 mV and 0.6 mM Ca^{2+} at +100 mV. Moreover the conductance-voltage relations showed that $V_{1/2}$ depended on intracellular Ca^{2+} concentrations. Ionic substitution showed that this current was anionic. Anion more permeant than Cl^- caused a shift of the activation curve toward more negative values, whereas less permeant anions caused an opposite effect. Finally application of blockers for CaCCs, such as niflumic acid and $CaCC_{ihn}$-A01, caused a reversible inhibition of the current. All these properties are similar to those of TMEM16A and/or TMEM16B, further supporting the hypothesis that these proteins are responsible for CaCCs in mouse VSNs. Additional studies are necessary to fully characterize the physiological function and regulation of these channels in VSNs.

Olfactory event related magnetic fields in humans

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Recent imaging techniques show different advantages and disadvantages. The advantage of magnetoencephalography (MEG) is the high time resolution in combination with source localization. The objective of the current study was to analyze the spatio-temporal patterns of human cortical activity during odor perception.
In a set of 12 normosmic healthy subjects (six male and six female; age range 19 – 28 years) we investigated the onset of brain activities in relation to ipsi- and contralateral stimulation with two odorants (phenyl ethyl ethanol [PEA] and hydrogen sulfide [H_2S]). Olfactory stimuli (duration 200 ms; ISI 40 s) were applied using an olfactometer (OM4, Burghart Instruments) without causing concomitant stimulation of mechano- or thermoreceptors. Brain activity during odor perception was recorded with a 248-magnetometers whole-head MEG system (MAGNES® 3600 WH, 4-D Neuroimaging) confined in a magnetically shielded room.
Olfactory responses were identified shortly (within 150 ms) after stimulus onset in both hemispheres. Stimulation of the ipsilateral side provided earlier signals compared to contralateral stimulation in the primary olfactory cortex, hippocampal gyrus (HC), parahippocampus (PHC), amygdala, and orbitofrontal cortex ($P < 0.001$). In the HC and PHC, we observed gender differences with regard to higher amplitudes in female subjects (factor "gender" $p=0.016$ and $p=0.021$). Also within the HC region, a lateralization to the left hemisphere was visible indicating for left shifted olfactory processing in this brain region (interaction "hemisphere" by "stimulation side" $p=0.019$). In addition, the more unpleasant odor (H2S) provided higher amplitudes in the entorhinal cortex compared to the more pleasant rose-like odor (PEA) indicating for potential hedonic encoding.
To our knowledge, this is the first study showing localizations of early olfactory brain activity in humans within 150 ms after stimulus onset. We suggest the MEG recording technique as a suitable tool to define the functional network of olfaction in healthy subjects and in patients with olfactory dysfunction.

Smell deficits as an endophenotype in patients with non-syndromic cleft lip and/or palate and their non-affected first-degree relatives: a pilot study

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Cleft lip and/or palate (CL/P) is one of the most frequent congenital birth defects with an incidence of 1/700 live births and a multifactorial etiology. Although recent studies such as linkage and association studies, have given insight in the genetic etiology of CL/P, most of the causal genes remain unidentified. A candidate gene approach, by the study of endophenotypes, is a unique and promising manner to reveal more of the genetic etiology of CL/P. Endophenotypes are characteristics that are associated with a condition and are considered to be an expression of the underlying susceptibility genes of this condition. One of the possible endophenotypes of CL/P is a higher frequency of olfactory dysfunction in patients and their non-affected first-degree relatives. Although olfactory function has not extensively been investigated in patients with non-syndromic CL/P, there is some evidence for a decreased capacity to smell in patients with CL/P and their non-affected relatives. Furthermore, olfactory dysfunction within syndromic CL/P could be an indication for reduced smell capacity within non-syndromic patients. When smell dysfunction is determined in non-affected relatives, it could be an indication for an underlying genetic cause.
In this pilot study with 48 patients, 41 non-affected relatives and 23 controls, smell capacity was tested using the Sniffin' Sticks (Burghardt), testing for smell threshold, discrimination and identification. Furthermore, a questionnaire for olfactory dysfunction was used to compare objective and subjective perception of the smell capacity. To confirm the central etiology of the smell deficits, an MRI was taken for volumetry of the olfactory bulb, expecting smaller olfactory bulb volumes in subjects with a decreased smell capacity. Structural defects were examined using acoustic rhinometry and rhinomanometry in subjects showing an olfactory deficit.
The pilot study revealed a significant olfactory dysfunction in patients with CL/P ($p=0.018$) and their non-affected relatives ($p=0.023$), compared to the control group. More olfactory dysfunction was seen in patients and relatives with a familial history of CL/P, indicating a genetic origin of this feature.
This study is the first to show decreased smell capacity in patients with CL/P and their non-affected first-degree relatives, indicating that olfactory dysfunction could be considered to be an endophenotype of non-syndromic CL/P.

An altered olfactory profile in children diagnosed with autism spectrum disorder

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The brain substrates implicated in autism spectrum disorder (ASD) are highly overlapping with the neural substrates of olfaction. Indeed, many anecdotal observations report altered responses to smells in children and adults with ASD. Here, we set out to test the hypothesis that olfactory responses are altered in children with ASD, and that these altered responses may serve early diagnosis. The sniff-response is a phenomenon whereby nasal airflow parameters are rapidly modulated in accordance with odorant content. For example, pleasant odors are automatically sampled with strong sniffs, yet unpleasant odors are sampled with weak sniffs. Thus, by measuring nasal airflow (sniffs) alone, we obtain a non-verbal measure of olfactory perception and processing. We developed a pediatric olfactometer that delivers pleasant and unpleasant odors and measures the concurrent sniff-response. A complete experiment consisted of 20 trials, half consisting of the unpleasant odor butyric acid or rotten fish, and half consisting of the pleasant odor phenyl-ethyl alcohol or herbal essence. Inter-trial Interval was 30 seconds, and total study duration was no longer than 15 minutes. The task involved mere exposure to the odorants, and measurement of the ensuing sniff. There were no task instructions to follow, and no active task to complete. Eighteen children diagnosed with ASD (17 boys, mean age 7.1±2.3 years), and 8 typically developed (TD) children (7 boys, mean age 6.9±2.3 years) completed a sufficient number of trials to allow analysis. Whereas TD children displayed a typical sniff-response, namely taking a larger sniff for the pleasant odor rather than the unpleasant odor (sniff volume: $t(7)=3.77$, $p<0.01$), children with ASD showed no such modulation, and often displayed a reversed sniff-response, favoring the unpleasant odor ($t(17)=0.24$, *NS*). In addition, within the ASD children there was a striking correlation of $R^2=0.83$ ($p<0.001$) between their composite sniff-response score (difference in sniff to pleasant vs. unpleasant odors) and their independently obtained autism severity scores (ADOS). Taken together, these results imply that altered olfaction in children with ASD manifests in an automatic non-verbal measure (sniff-response) that may have diagnostic value.

Analysis of human TAS2R variations underlying individual difference in the food preference and bitter taste evaluation

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Bitter taste is one of the important factors that affect food preference and palatability. It is detected by various TAS2Rs expressed in the bitter taste cells of the taste buds. However, the evaluation of the bitterness of the food is subjective and divergent depending on the person. For example, genetic variations in TAS2R38 gene lead to the corresponding amino residue substitutions and affect bitter taste sensitivity to phenylthiocarbamide (PTC) or 6-n-propylthiouracil (PROP). However, in most cases the genetic factors that may affect the bitter sensitivity of an individual are not yet well understood. In this study we asked how individual subjects evaluate the bitterness of protein foods or vegetables by a questionnaire study or by a quantitative sensory test. We then analyzed TAS2R polymorphisms by sequencing the genome of the subjects. Data from a total of about 150 university students were used for this analysis.

Correlations between the questionnaire, the sensory test and the TAS2R polymorphism were systematically analyzed. For example, we found that individual variations in the bitterness intensity of caffeine in the sensory test had a significant correlation with bitterness of the tea and also with the average frequency of drinking coffee in the questionnaires but no correlation was found with any TAS2R genotypes that we analyzed. Since oligopeptides are major bitter compounds in protein foods and there are some TAS2Rs that are activated by them, some bitter dipeptides were then analyzed. We found that individual difference in the bitterness of Gly-Phe may be correlated with a single nucleotide polymorphism (SNP) in TAS2R1 that are known to be involved in the reception of some bitter oligopeptides including Gly-Phe. We also found that subjects with higher bitter sensitivity for grapefruit significantly perceive the bitterness of Gly-Tyr more strongly, suggesting that Gly-Tyr and the unknown bitter grapefruit components may share the common bitter receptor. On the other hand, bitterness involved in the cruciferous plants was not correlated with any of the genetic variations in the oligopeptide TAS2Rs. Unlike synthesized bitter chemicals, natural foods may contain divergent bitter substances. The genetic analysis of the individual difference in the bitter perception will be more difficult since multiple TAS2Rs may be potentially involved. However, since the genetic variations in the taste perception of the natural foods are based on the human evolution, it may provide new clues for identifying the relation between natural bitter ligands and the bitter receptor TAS2Rs.

SATIN: SATiety INnovation – Development of an in vitro platform to identify improved satiating food components

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The worldwide predominance of obesity almost doubled between 1980 and 2008. According to country estimates for 2008, over 50% of both men and women in the WHO European Region were overweight, and roughly 23% of women and 20% of men were obese. SATIN – SATiety INnovation is an EU/FP7 funded project, which aims to develop new food products using the latest processing innovation techniques. Exploiting better understanding of the biological processes in the stomach and the brain that underpin what induces satiety, the project will evaluate whether this approach is a viable weight management tool. SATIN represents a consortium of 18 partners from 9 European countries including research institutes and SMEs and large companies which specialize in novel food formulation and production. SATIN is divided into seven different work packages (WP) and AXXAM is leader of WP1. The overall objective of SATIN is to use novel food processing technologies to alter the structure of foods to accelerate satiation, enhance satiety and to reduce appetite. In a first phase the SME and industry partners developed novel food processing technologies combining optimized foods structures and active ingredients to enhance satiation / satiety. The aim of WP1 is to evaluate the likely impact of these novel foods by *in vitro* modeling of the gastrointestinal tract using dynamic gut models and automated screening assays including gastrointestinal chemosensory receptors and hormone secretion pathways. This *in vitro* work will then be validated in *in vivo* studies of biomarkers of appetite. In WP1, an *in vitro* screening platform was developed which allowed verification of the effects of food ingredients on gastrointestinal hormone secretion and chemosensory activation. This screening platform consisted of primary assays based on enteroendocrine cell lines, which endogenously express receptors for food components and the hormone production and secretion machinery, and recombinant reporter cell lines expressing the receptor for the specific gastrointestinal hormone and a functional readout. Thus, enteroendocrine cells and recombinant reporter cell lines were functionally coupled, to quantify the gastrointestinal hormone secretion by mean of the reporter gene activation. In addition, secondary recombinant assays have been established to directly investigate the effect of food derived complexes on chemosensor receptors like GPR120, GPR43, GPR93, and TGR5. The effect of isolated food ingredients and of matrices containing the food ingredients subjected to intestinal digestive/fermentative process, through a dynamic gut model (the SHIME technology platform) were tested on the in vitro platform. Different activity profiles were obtained and some examples will be shown.

Activation of the newborn rabbit's olfactory bulb and piriform cortex by configural versus elemental odour mixture

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Mammals are surrounded by a huge number of chemically complex odours, even early in life. In some mixtures, only the component odours are perceived (elemental perception) while in others a new odour percept (configuration) emerges (configural perception). In the rabbit, newborns display sucking behaviour in response to the mammary pheromone, which also promotes rapid learning of new odorants by associative conditioning. We previously showed that after conditioning to ethyl maltol (odorant B), pups respond to B and the A'B' mixture (32/68 ratio), but they do not respond to odorant A (ethyl isobutyrate) or the AB mixture (70/30 ratio). This suggests configural and elemental processing of the AB and A'B' mixtures respectively. Here we hypothesized that involvement of brain regions such as the olfactory bulb (OB) and piriform cortex (PCx) might differ depending on the mixture processing.

Pups were conditioned or pseudoconditioned to B on postnatal day 3 and their reactivity was examined on day 4 after exposure either to B, AB or A'B'. Conditioned (B, AB, A'B') and pseudoconditioned pups (PC_B, PC_{AB}, $PC_{A'B'}$) were then processed 90 min after odour presentation for Fos immunohistochemistry to map OB and PCx activation.

Concerning PC groups, the granular layer of the OB and the PCx were more activated in PC_{AB} versus $PC_{A'B'}$ pups and the granular pattern was more contrasted between PC_{AB} and PC_Bthan between $PC_{A'B'}$ and PC_B pups. Conversely, in conditioned groups, the PCx was less activated in AB versus A'B' pups. Comparison of PC and conditioned groups in OB and PCx indicated higher activation in A'B' than $PC_{A'B'}$ pups while AB pups showed lower activation than PC_{AB} pups.

These data support a differential brain processing of both mixtures. The ratio of components in AB may lead to different information reaching the OB compared to A'B'. Consequently, the PCx may build a modified representation of the stimulus, based on peripheral afferent activity from olfactory mucosa and bulb, and contributes to the perception of the AB odour configuration in addition to the odours of the elements. Differential involvement of reciprocal connections between OB and PCx may also contribute to differences between the mixtures. Importantly, differences occurred both in pseudoconditioned and conditioned animals. Together with recent behavioural data, this highlights that configural perception happens even in relatively immature animals, and points out the newborn rabbit as a valuable model to further explore odour mixture processing from molecules to brain and behaviour.

Nutritive versus non-nutritive sweetener preference and reward in mice

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It is commonly assumed that sugars are more rewarding than non-nutritive sweeteners because of their caloric value. However, isocaloric sugars differ in their postoral reward effects, with glucose and sucrose much more rewarding than fructose in rodents. We therefore compared the preference of mice for 8% sucrose, glucose, and fructose solutions versus a non-nutritive sweetener (0.1% sucralose + 0.1% saccharin, S+S). Brief (1-min) two-bottle choice tests indicated that C57BL/6J (B6) mice preferred S+S to all three sugars. Yet, in 48-h choice tests, which are influenced by postoral effects, B6 mice significantly preferred sucrose and glucose to S+S, but S+S to fructose. In progressive ratio lick tests, B6 mice licked more for glucose but less for fructose compared to S+S. To evaluate strain differences in sweetener preferences, sugar vs. S+S choice tests were conducted with FVB/NJ mice, a sweet-sensitive strain like B6 mice. In an initial 48-h test, the FVB mice strongly preferred S+S to fructose, but after separate sweetener vs. water tests, the mice preferred fructose to S+S. They displayed an even stronger preference for glucose over S+S and also preferred glucose to fructose. These findings indicate that post-oral fructose is more rewarding to FVB mice than B6 mice, while glucose has similar effects in the two strains. Together these findings indicate that long-term sugar vs. sweetener preferences are based on postoral reward, not on sweet taste or calories per se. Furthermore, just as there are mouse strain differences in sweet taste sensitivity there are also strain differences in the postoral reward actions of sugars.

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Assessment of peripheral olfactory nerve image in patients with idiopathic olfactory impairments in comparison to healthy volunteers

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Objectives: To show the reduced nasally administered thallium-201 transport to olfactory bulb in patients with idiopathic olfactory impairments in comparison to healthy volunteers.

Methods: 10 patients with idiopathic olfactory impairments (5 women and 5 men; 23–73 years old; T&T recognition thresholds, 5.7 ± 0.5) and 10 healthy volunteers (3 women and 7 men; 35–54 years old; T&T recognition thresholds, 0.7 ± 0.6) were enrolled in the study. The causes of olfactory dysfunction in the patients were unknown. Thallium-201 was administered unilaterally to the olfactory cleft, and SPECT-CT was conducted 24 h later. Separate MRI images were merged with the SPECT images.

Results: Nasal ^{201}Tl migration to the olfactory bulb was significantly lower in the patients with idiopathic olfactory impairments than in healthy volunteers.

Conclusions: The peripheral olfactory nerve is reduced in the majority of patients with idiopathic olfactory impairments. It is difficult to directly view the peripheral olfactory nerve with current magnetic resonance imaging. Assessment of the ^{201}Tl migration to the olfactory bulb is the new method for the evaluation of the olfactory nerve in patients with idiopathic olfactory impairments.

Olfactory brain responses in congenital anosmia

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Congenital anosmia (CA) is diagnosed following a total lack of current or past olfactory perception. We asked whether this subjective lack is associated with an objective lack of odor responses. A powerful implicit objective measure of olfaction is the sniff-response, namely odor-specific sniff patterns. Thus, we measured sniffing in 8 (5F) normosmics and 7 (4F) anosmics concurrent with odor pleasantness estimation of vanillin, ammonium sulfide and odorless air (8 trials per odor). An ANOVA on sniff duration with conditions of *osmia*(normosmic/anosmic), and odor (clean/pleasant/unpleasant), revealed no main effect of osmia ($F=1.21$, $p = 0.29$), reflecting that normosmics and anosmics sniffed at equal duration, a main effect of odor ($F=24.21$, $p < 0.001$), reflecting shorter sniffs for unpleasant vs. pleasant odors, and no interaction ($F=2.2$, $p =0.131$), implying that normosmics and anosmics sniffed similarly. Post-hoc tests indeed revealed a significant reduction in sniff duration for unpleasant vs. pleasant odors in both normosmic ($t=-4.0$, $p < 0.005$) and anosmic ($t=-3.8$, $p < 0.008$) subjects, which persisted despite total lack of anosmic odor awareness. Given this indication of implicit odor processing in CA, we next used fMRI to measure odor-induced brain responses in these individuals. An olfactometer delivered 4 different odorants (isovaleric acid, eucalyptus, PEA and ammonium sulfide) in an event-related paradigm with 11 normosmic (5F) and 12 anosmic (4F) subjects inside a 3-Tesla Siemens MRI scanner. Multi-subject analysis contrasting clean > rest using a GLM with random effects revealed similar patterns of sniff-evoked brain activation in olfactory associated brain regions in normosmics and anosmics. Moreover, region of interest analysis in the Inferior Frontal Gyrus (IFG), an area identified in previous studies as that most activated in odor identification, revealed significantly higher activation for unpleasant vs. pleasant odors in anosmics (mean % change pleasant = 2.2, unpleasant = 2.8, $t(11)=2.43$, $p=0.03$), despite no concurrent awareness for odor. In sum, individuals diagnosed with CA, who had not consciously perceived an odor in their remembered life, nevertheless had implicit sniff-responses to odors, sniffing alone activated their brain substrates of olfaction, and their IFG responded differentially to pleasant and unpleasant odors. Taken together these results depict a brain response to smell in CA. These findings provide promise for treatment in CA, as perhaps we can teach these individuals to sense what their brain already knows. Finally, the difference in brain response between anosmics and normosmics may point to brain mechanisms of conscious awareness.

Taste sensitivity of larval styloconic sensilla mediates the discriminating capability between different bitter compounds in two Papilionid species.

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The taste discriminating capability of bitter compounds is particularly useful for herbivorous animals because plants produce noxious compounds in their nutritious tissues, some of which are toxic while others are only unpalatable. On this basis, we examined the role of the peripheral taste system on the discriminating capability between different bitter compounds, by using the larvae of Papilio hospiton and Papilio machaon as experimental model: two oligophagous lepidopteran species phylogenetically related, but presenting different food choices. We had previously characterized three bitter-sensitive GRNs in the lateral and medial styloconic sensilla in the larval maxillae of both species: two in the lateral and one in the medial sensillum.

The spike activity from these neurons was recorded by the tip-recording technique following stimulation with several bitter taste stimuli: two toxic compounds (nicotine and caffeine), and two unpalatable compounds (salicin and quercitrin). The results show that nicotine and caffeine activate all bitter-sensitive GRNs, while salicin and quercitrin only two of them, one in the lateral and one in the medial sensillum.

We also did experiments to study the feeding behaviour, in response to each of the four bitter compounds. The results show that larvae eat glass-fiber disks moistened with salicin and quercitrin, while rejecting those with nicotine and caffeine. Afterwards, lateral sensilla were ablated in the same larvae and behavioral tests were repeated with nicotine and caffeine. The results show that larvae also eat the disks with the toxic compounds.

In conclusion, these results support the hypothesis that P. hospiton and P. machaon larvae are able to discriminate between different bitter compounds by means of a labeled-lines code and that their discriminating capability seems to be mediated by the sensory input from GRNs of the styloconic sensilla.

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

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Olfaction is based on the chemosensory detection of an apparently unlimited number of volatile compounds by the olfactory sensory cells. The response spectrum of each sensory cell is determined by its odorant receptor type. The large number of structurally diverse receptor subtypes and their selective but non-specific ligand spectrum is considered as the basis for the perception of myriads of odors by combinatorial coding. Receptors of the OR37 subfamily differ from these principles; this small group of receptor types is structurally conserved and only present in mammals. In the search for special mammalian specific ligands, long-chain aliphatic compounds were considered as candidates because they are present in secretions of mammalian skin glands. Due to the hydrophobicity of such molecules it was necessary to establish a novel experimental approach to monitor and to characterize an activation of receptors. Employing the optimized approach it could be shown that long-chain aliphatic aldehydes activate cells that express OR37 receptors. Subsequent analyses revealed that the OR37 receptors seem to be quite specific; different receptor subtypes discriminated aldehydes that differ in chain length by only one C-Atom. To approach the question whether these OR37 receptor-activating compounds are in fact produced and emitted by mice themselves, chemical analyses of various secretions were performed. Gas chromatographic analyses in combination with mass-spectrometry revealed that hexadecanal, the ligand of the OR37B receptor, is present in feces of mice. Further analyses support the notion that hexadecanal is an ingredient of secretions produced by anal glands to cover the surface of feces; thus, it could act as a chemosensory signal. Analyses of probes originating from the skin of animals revealed that it contained compounds that activate receptor type OR37C. Subsequent studies indicate that the activating compounds are not constantly present on the skin but rather are secreted when the mice are placed in a novel environment. Further studies will be necessary to explore whether the secretion of OR37C activating compounds is part of a scent marking behavior or if it is secreted as response to a stress reaction.

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Odor Hedonics Related Brain Activation: An fMRI Study

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Odors are often thought to bring about desirable effects on human psychological and physiological states. Some odors can give rise to emotional or mental responses, positive or negative thoughts, occasionally leading us to recall affective memories with a strong feeling. It is widely acknowledged that the information of a smelling sense is projected from the olfactory bulb to the piriform cortex and the amygdala, and this physiological underpinning shares, together with the orbitofrontal cortex (OFC), neural mechanism selectively involved in emotion and associative learning. Given this, we tried to shed light on brain regions which might be preferentially engaged with the processing of hedonically tinged odors.

In this neuroimaging study, a total of eighteen Japanese female subjects participated in an functional magnetic resonance imaging (fMRI) experiment. Each of the subjects was provided five odors (i.e., Vanillin, Thesaron, Rose Abs., Iso Bornyl Acetate, and DPG) in a random order without repetition in each run. This block design presentation of items repeated six times so that the overall runs gave a total of thirty critical trials. For each trial, they were requested to focus attention to each smell stimulus delivered thorough a face mask during 200 seconds, and take a rest for 120 seconds as an inter-stimulus interval. The scanning was performed using an echo planar imaging sequence with a 2000ms repetition time (TR). Posterior to the fMRI session, responses to a questionnaire about the off-line evaluation of the odors were collected from the subjects, and this enabled us to run a maximum-likelihood factor analysis with a Promax rotation (exclusive of DPG as a neutral baseline stimulus). By virtue of this post-hoc analysis, the critical stimuli were classified into two groups, which are a positive mean factor score group (Vanillin and Rose Abs.), labeled here as "pleasant", and a negative one (Thesaron and Iso Bornyl Acetate) unified as "unpleasant". A univariate analysis of General Linear Model was conducted by using SPM8 to extract significant areas of activation for this contrast, and the t statistic of the "unpleasant" versus "pleasant" items revealed significant peak voxels in ventral and medial regions. Although our result is not conclusive but considered preliminary, the vicinity of these maxima effects to left caudate, right hippocampus and left thalamus yields insights to the significance of the affective valence and the cognitive control effects triggered by specific olfactory stimuli in a hedonic context.

Electrophysiological and behavioral analysis of Drosophila taste perception by selective inactivation of the gustatory receptor neurons

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Physiological analysis have shown that a single taste sensillum, the gustatory organ of the fly, consist of basically four receptor neurons; sweet-, low salt-, bitter/high salt- and water-sensitive neurons. Recent molecular analysis of the gustatory receptors (GRs) in *Drosophila* also showed that divergent but exclusive subsets of GRs are co-expressed in a single sweet or bitter neuron, supporting that the two taste modalities are independent. However, since the expression of GRs and other taste receptor molecules in varous taste neurons and organs are unexpectedly divergent, it is not yet clear how many taste modalities are encoded in the fly taste system.

To investigate how physiologically or molecularly defined taste neurons contribute to the perception of a taste modality, we investigated two *Drosophila* transformant flies in which the labellar *Gr5a* -expressing sugar neurons or *Gr66a* expressing bitter neurons are selectively inactivated by an ectopic expression of an apoptosis gene *reaper (rpr)* under the control of*Gr-GAL4* drivers. The electrophysiological analysis of the labellar taste sensilla showed that different neuron(s) are inactivated depending on the sensilla types as well as the *GAL4*driver. The flies were then subjected to feeding tests for various taste ligands to know if any behavioral changes are associated with the neuronal inactivation. In this study we show how physiologically or molecularly defined neurons control the feeding behavior and will discuss how different taste modalities are encoded by differrent taste neurons.

Determination of the cortical current sources generating the olfactory-induced EEG voltage changes using sLORETA and EEGLAB ICA/DIPFIT2 analysis of 32 electrodes recordings.

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Olfactory event related potentials (OERP) are recorded by averaging EEG recordings. However, the brain cortical activity generating this scalp recorded activity is not yet well understood. In this study we performed two types of analysis to estimate the location of the underlying cortical current sources.
EEG activity (Biosemi Active Two system, 32 active electrodes placed according to the international extended 10-20 system; 256 Hz sampling rate; band pass 0.5-40 Hz) was recorded from healthy subjects performing an odor detection task with closed eyes. The odorant was delivered during 200 ms in one nasal cavity and this stimulation was followed, 2.6 later, by an imperative auditory stimulus during which the subject had to press a button to indicate whether he/she had perceived the odorant or not. The EEG data were processed using Matlab with the EEGLAB toolbox. EEG artifacts were removed by independent component analysis (ICA). Olfactory event-related potentials (OERP) were obtained using the onset of the olfactory stimulation as time reference. The OERPs were then analyzed by the sLORETA software (standardized low-resolution brain electromagnetic tomography (Pascual-Marqui, 2002) to calculate the standardized current source density at each of 6239 voxels in the gray matter and the hippocampus of the MNI-reference brain. This method is based upon a linear weighted sum of scalp electrical potentials and estimates the underlying sources assuming that neighboring voxels should have a maximally similar electrical activity. In addition, the brain sources underlying the OERPs were also determined using the EEGLAB independent component analysis and the location of relevant dipoles using DIPFIT 2 analysis (Delorme and Makeig, 2004).
In a first step we checked the accuracy of the dipole location methods by examining the evoked potential when the subjects press the button with the right hand: a specific activation of the precentral and postcentral gyrus was observed in the left hemisphere; this activation started about 50 ms before pressing the button (initial phase) and increased further during 100 ms (late phase) as the button was kept pressed. As expected, the initial phase was not observed in these cortices in the right hemisphere; the late phase was present but much smaller. The activity in the left motor/sensory cortex area was also observed using EEGLAB ICA/DIPFIT2 analysis.
Given these positive controls, we analyzed the OERPs recorded from 5 subjects. Time series of the activity in several brain regions of interest in each hemisphere (insula, parahippocampal , anterior cingulate, posterior cingulate, frontal cortex, occipital cortex etc.) will be presented.
We suggest that these methods of brain imaging, with a high temporal definition, would be an interesting complement to fMRI analysis of olfactory-evoked local brain activities.

Identification of a subset of human olfactory receptors that respond to sweat carboxylic acids.

Alex Veithen, Sebastien Patiny, Françoise Wilkin, Magali Philippeau, Pierre Chatelain

Small carboxylic acids constitute a class of odorant molecules generally perceived as unpleasant. Several of these acids are major components of the human sweat and are important targets for anti-axillary malodor programs. Despite the fact that a few studies have reported the identification of human olfactory receptors for small carboxylic acids, the mechanism of their perception remains to be understood. Here, we describe the identification and functional characterization of 11 human ORs that are activated by small carboxylic acids. When tested in a HEK293 cell-based expression with a luciferase-based gene reporter assay, all these receptors are found to respond to at least one acid of the human sweat. Receptors activated by the most prominent malodor acids of the sweat, such as 3-hydroxy-3-methylhexanoic acid, trans-3-methyl-2-hexenoic acid and isovaleric acid, have been identified. Structure-activity relationship studies have revealed that the range of selectivity may vary from one receptor to another, with one half of the ORs responding to one or two agonists while the other half responds to 5 or more molecules.
Interestingly, all the identified receptors belong to class I ORs, also known as fish-like. This subfamily of receptors is assumed to be less derived than the class II, which expended during airborne vertebrates evolution. Our results support the hypothesis of an implication of class I ORs in the perception of small fatty acids and more particularly to those present in the human sweat.

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Evaluation of pleasantness of odorants in different European countries

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Introduction: Evaluation of pleasantness of odorants is fast and simple for the patients. Interindividual and international differences of pleasantness ratings of odorants remain main trouble in case of use of such test of olfactometry in clinical practice. The goal of the study was to evaluate pleasantness of odorants in different European countries.

Materials and Methods: We included 247 (159 women) healthy subjects of average age 29.4 years. In total 47 subjects were examined in the Czech Republic, 43 in Germany, 40 in France, 75 in Italy and 42 in Slovakia. All subjects were tested using New Test of Odour Pleasantness, which consists of 32 hedonically strong odorants; some of odorants were also strong trigeminal irritants. Subjects had to choose, whether odorant was pleasant, neutral, unpleasant or very unpleasant. Standard test of olfactometry was used as well (Odourized Markers Test or Sniffin‘ Sticks test – part Identification).

Results: Hedonic mostly preferred characteristic was the same in 16 of 32 odorants in all tested nations. Further, in other 7 odorants there was equal evaluation of category pleasant, neutral and unpleasant. Especially lemon, vanilla, men’s and women’s perfume, fish, propyl acid, valeric acid, caproic acid and octanoic acid were similarly evaluated.

Conclusion: Evaluation of pleasantness of odorants would be of alternative test to standard ones based especially on identification. Despite the differences in hedonic evaluation of some of odorants, some of them were equally categorized across all investigated nations.

Determining the Gender of an Odorant

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Masculinity and femininity are thought by perfumers to be central dimensions of olfactory perception (Zarzo 2008). Since molecules have no natural gender, this raises the question of how people make these gender determinations. Are the physical aspects of an odorant stereotypically associated with gender (semantic gender) driving these decisions? Or is it possible that the structure of the participant's language plays a role? So, do people who speak languages that employ grammatical gender classify odorants in a way that is more consistent with their grammar than others, simply because of the linguistic convention? The grammatical gender of objects is functionally independent of their semantic gender (Aronoff, 1994), yet some literature suggests that thinking of gender for speaking has an effect on object perception (Boroditsky, Schmidt & Philips, 2003). This study addresses whether this is the case in olfactory gender by examining the way that 29 people (16 French/English bilinguals and 13 English speakers) described 16 odorants. The odorants were selected such that eight of them carried the same semantic gender in English (determined via pilot) as the French grammatical gender, while the other odors diverged in this regard (divergent genders). The odorants were presented to participants in bottles that were labeled with the name in English. All testing proceeded in English, and participants were unaware as to the purpose of the study. Each of the participants described the odorants by providing three adjectives to describe the odorant. Each of the adjectives generated were later evaluated as masculine (-1) or feminine (+1) by five additional English speaking participants who were naïve as to the way that the words were collected. These ratings were summed for each word and then each odorant. Comparisons were made of the difference between grammatical gender maximums and implicit ratings in the divergent gendered odorants for the two groups of speakers. Results showed that showed that the responses from the bilinguals were closer to grammatical gender [$t(25) = -2.13$, $p = .02$, one-tailed] and more variable [$F(15, 12) = 2.63$, $p = .05$, one-tailed]. Similar comparisons using the amount of difference from semantic gender showed no differences between the two groups of speakers. These results support cross-linguistic differences in thought, at least at the level of linguistic representation. They suggest that thoughts of olfactory gender are influenced by language structure rather than simply due to aspects of associations to physical properties of the scent.

Compared analysis of dimensions in the human odor perceptual space

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Despite ongoing research in this field, the basic principles of the odor perceptual space are still not well understood. So far, various studies using dimensionality reducing methods like Principal Component Analysis (PCA) or Multidimensional Scaling came to one consistent finding: The principal axis of odor perception is organized from “unpleasant” to “pleasant”[1, 2]. but obviously, odor perception is a more complex process [3], thus the consequent next step will be to learn about other categories that shape odor perceptual space. A recent study applied Non-Negative Matrix Factorization (NMF) to the odor profile database from Dravnieks [4] and proposed an organization along 10 categories [5]. Interestingly, the complexity of this categorical model of human perception correlates very well with previous findings of 9 functional modules derived from 2DG uptake images of early processing input patterns in the rat olfactory bulb.

As Dravnieks data consists of only a limited number of about 140 compounds, we applied NMF to a significantly larger database (odor profile catalogue of the Aldrich company [6]) consisting of 850 compounds described by 171 descriptors. We found a set of 14 stable basis vectors, the random error suggests a general solution of about 10 categories. While both results differ in some of their categories, especially the dimensionality estimation stays roughly the same.

Our results suggest that the proposed estimate of 10 categories by Castro et al. can be roughly reproduced by the much larger Aldrich data. Furthermore, at least 3 basis vectors and their corresponding descriptors correlate very well to functional clusters from the rat olfactory bulb, which might be evidence for a similar developing of early olfactory processing between rodents and humans. We conclude that human odor perception space might be described by a representation using the proposed 10 dimensions.

Although the exact meanings (i.e. odor qualities) of these categories might be subject for further investigation, there are some basic categories (fruity, sickening and nutty) that appear to have been conserved in the course of evolutions in human.

Odor-dependent enhancement by estradiol of neural activity in accessory olfactory bulb of female mice.

Saori Yano

In many animals, body-originated odor is used as cues for mate choice. For instance, female mice obtain cues about male mice from their urine, resulting in a selection of mating with genetically different partners. In our previous study, we found that BALB/c females during the period from metestrus to proestrus prefer the urine of genetically dissimilar males (C57BL/6) over that of similar males (BALB/c), while they did not show any selection in estrus when the level of estradiol is the lowest. We also showed that this odor selection is dependent on estradiol. Such a periodical change is likely to be advantageous to females, as the possibility to encounter genetically different males would be increased with the consequence that the females have offspring with genetic heterogeneity.
In the present study, we explored the further detailed mechanism of the estradiol-dependent selection. First, to examine whether vomeronasal system is involved in, we conducted behavioral tests using vomeronasal organ-removed (VNX) females. Sham operated females showed estrus stage-dependent selection, but VNX females showed no selection throughout all stages. These results suggest that the vomeronasal system is necessary for the odor selection. Second, we performed immunohistochemical staining of c-Fos, a marker of neural activity, in order to detect regions that can be related with estradiol-dependent odor selection in the vomeronasal system. Chronic administration of estradiol increased the number of c-Fos-IR cells in both the granular cell layer and the mitral cell layer in the accessory olfactory bulb after exposure to urine of genetically similar males, but not to that of dissimilar males or distilled water. These trends were not observed in the main olfactory bulb. Based upon the results, we propose following ideas; (1) estradiol is involved in the regulation of odor selection-related neural activity at the level of the vomeronasal organ or the accessory olfactory bulb, (2) urine from genetically different males plays no roles in attracting females. On the other hand, urine from similar males rather repels females.

Peripheral olfactory system is impaired in preclinical stage of Alzheimer's Disease Mouse Model

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Olfactory dysfunction is a common symptom in neurodegenerative diseases including Alzheimer disease (AD). Recently, it has been suggested that olfactory dysfunction may be a potential biomarker in assessing either onset or progression of AD. Although there is some clues in involvement of peripheral olfactory organs in olfactory dysfunction along with AD pathology, the underlying events has not been fully understood. From behavioral tests, we found that transgenic AD model mice, overexpressing amyloid precursor protein, have olfactory impairment in presymptomatic age without failure on formation of spatial memory. Their olfactory epithelium exhibited significant decrease on both thickness and cell number with massive neuronal death and enhanced formation of oligomer of amyloid-β (Aβ), especially Aβ*56 (12-mer). In addition, treatment of Aβ oligomer mediated cell-autonomous degeneration in vitro cultured olfactory receptor neurons o P38 MAPK activation. Furthermore, dopaminergic neurons in periglomerular region of olfactory bulb were also decreased, indicating impaired integrity of olfactory neural circuit between peripheral and central olfactory system. Taken together, we suggest that the impairment of peripheral olfactory system is a presymptomatic event in AD and thus can be potentially adopted as a useful biomarker for early AD diagnosis.

Seeing Odors in Color: Cross-Modal Associations in 5- to 10-Year-Old Children from Two Cultures

Nathalie Goubet

The goals of the study were to explore the possibility that school children form consistent cross-modal associations between odors and colors, and to document possible developmental changes. We also aimed to evaluate the extent to which such associations are based on the semantic identification of odors and/or on their perceived cultural familiarity. Participants were French (F) and American (A) school-age children ranging from Kindergarten to 5th grade (N = 385, 52% girls). Children smelled 9 odorants [culture-specific: grenadine, lavender, methyl salicylate (root beer), maple syrup; non-culture-specific: strawberry, butyric acid (cheese), chamomile, bergamot, blank] and had to choose a matching color from an 8-color wheel (purple, red, blue, yellow, green, white, orange, brown), followed by hedonic rating, and identification. Three-way log-linear analyses (Country x Age x Color) and follow-up chi-square tests revealed significant consistent odor-color associations for each odor. In particular, children showed associations consistent with their respective cultures (F children: purple-lavender, red-grenadine; A children: white/brown/green-methyl salicylate, p's < .05). Non-culture-specific odors were also significantly associated with colors (e.g., strawberry-red/orange/yellow, bergamot-yellow/orange). The pattern of odor-color associations did not change with age. A 9 odorant x 6 grade x 2 country mixed ANOVA on odor identification (ID, scores ranged from 0 to 3) yielded an odor x country interaction, p < .001. ID scores for root beer were higher for A children but ID scores for grenadine and lavender were higher for F children, p's < .001 (no difference for maple syrup). A main effect of grade was found, p < .001, the oldest age group (5th graders) having significantly higher ID scores than all other children, p's < .05. Finally, a main effect of odorant p = .001 indicated that ID scores were significantly higher for bergamot and strawberry, and lowest for chamomile, p's < .001. Chi-square tests of independence showed that odor-color associations differed between children who identified odors and those who failed to identify them, although even those who failed to identify an odor still made consistent color associations (p's < .05). In conclusion, 5-to-10 year-old children, like adults, make consistent non-random odor-color associations. These associations depend in part on exposure effects in the local culture and become more accurate with explicit semantic identification

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Promiscuity of Bitter Taste Receptors

Promiscuity of Bitter Taste Receptors

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Bitter taste can be considered a sentinel which provides protection against poison consumption, but it also makes many healthy food ingredients aversive to the consumer. Many clinical drugs suffer from low patient compliance due to their bitter taste. Recently, bitter receptors (TAS2Rs) were shown to be expressed extraorally and emerge as novel targets for infectious and respiratory disorders. Better understanding of molecular recognition of bitter compounds may provide improved tools for rational design of functional foods, help discover novel potential drugs, and provide a rational way for designing drugs with acceptable taste and higher compliance rates.

TAS2Rs belong to the family A of G protein-coupled receptors (GPCRs), one of the major drug target families. Intriguing differences between family A GPCRs (for which more antagonists than agonists are known) and TAS2Rs (for which agonists abound but antagonists are hard to find) are investigated. Recent breakthroughs in crystallography lead to experimental structures of about 20 different GPCRs which provide structural information about ligand-protein interactions and activation mechanism. Previous chemoinformatics and modeling studies found that GPCR promiscuity towards their antagonists correlates with features of the orthosteric binding site.

Here we performed a statistical analysis to unravel the features that determine TAS2Rs' promiscuity towards their agonists. Since no structure is yet available for TAS2Rs, we used descriptors related to the amino acid sequence of human TAS2Rs and looked for their correlation with the receptive range of the receptors. We found that the promiscuity correlates with the properties of residues present in their binding site. Our findings highlight the protein features responsible for promiscuity in the family of bitter receptors providing new venues for predicting and rationally modifying TAS2R selectivity towards their ligands.

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