



# 15th Meeting on Chemical Signals in Vertebrates

## Programme and Abstracts

3 - 5 November 2021

On-line conference



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## Foreword

Dear colleagues and friends, we are happy to welcome your “visit” for the **15th Meeting on Chemical Signals in Vertebrates**. Participants from twenty countries from the five continents will commute – electronically – toward the meeting base located in Dijon, Bourgogne, France.

The COVID-related pandemic has unfortunately disrupted the triennial pace of the CSiV meetings, so that the 2020 edition had to be postponed to 2021. Furthermore, due to persistent instability of the health situation in our countries, meeting in person had still to be temporarily forgotten. We had to mount a substitutive on-line solution.

Our option for a virtual meeting relies on an Internet platform (Gather Town) that playfully, but seriously, attempts to simulate watching both live and pre-recorded sessions. Between sessions, this platform will also allow quasi-live discussions among multiple interactants (for example, around posters). **Please, attentively consult the tutorial passed on to all participants before the meeting to become experts in timely navigating through the virtual conference setting, with its lobby, conference hall, and poster space!**

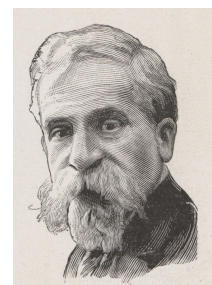
What is highly pleasing despite the situation, is that our informal network has resisted this viral adversity and many of us, senior fellows as well as many junior researchers, have proposed sharing their recent novel findings or stimulating syntheses in talks and posters. In addition, five symposia, and a panel of plenary and keynote talks by renowned speakers will make this meeting especially exciting.

Last but not least, our small but assiduous CSiV community has the privilege to receive encouragement from Dietland Müller-Schwarze, one of the founding fathers of semiochemistry and of this series of meetings.

We look forward to seeing you “in” Dijon!

Benoist Schaal and Matthieu Keller

*About the logo of the 2020 (2021) edition of CSiV:* As a favorite model species of the Dijon group, the “Leaping rabbit” was selected as the (admittedly mammal-centric) emblem of the 15th CSiV Conference. It is one of the numerous lively depictions of vertebrates realized by the well-known Burgundy’s sculptor François Pompon (1855-1933). We would have liked you to caress Pompon’s art in real life, while sipping a glass of Gevrey-Chambertin or Romanée-Conti in the Musée des Beaux Arts in Dijon....



## Ouverture

# CSiV 15: Widening Horizons

Dietland Müller-Schwarze

College of Environmental Science and Forestry,  
State University of New York, Syracuse, New York, USA

Organizers and contributors deserve a large round of applause for their hard and patient work to pull off this virtual meeting after the long pandemic-caused delay! Originally conceived as a one-off gathering 45 years ago, this 15th meeting paints a picture of a fuller understanding of the complex web of chemocommunication: We have come a long way from the enthusiastic - but shown to be naive - starting point, inspired by insect research: one compound was expected to trigger and control behavior in a very specific manner.

Our field has broadened to new horizons: besides multicomponent cues, we now learn about multisource and multifunction chemical signals. The range of study animals and settings has become richer, and we have learned enough that practical applications are becoming realistic.

***Multimodal communication and multisource semiochemicals.*** We now know that *multimodal* communication is commonplace: Within the chemical senses, VNO and MOS may both be involved. Chemical and visual cues can play a role, as in raptors (Potier et al.); olfactory and acoustic signals help sea lion mothers to recognize their pups (Pitcher et al.). Human infants are better able to rapidly categorize the mother's face if maternal body odor is present (Kiseleva et al.). In *multisource messaging*, the rabbit mammary pheromone also occurs in anal gland secretion (Moncomble et al.). In sifakas, genital and sternal secretions, urine and tree gouging allow for a "flexible olfactory syntax" with an incredible range of messaging (Drea and Greene).

***Range of settings and behaviors studied.*** We are widening our horizon in terms of *different settings* to study animals. Comparing wild and captive animals can yield surprising results: In tamarins, free-ranging animals had a richer mixture of compounds in their secretion (Poirier et al.). Water voles

are attracted to male urine odor in both an animal facility and in the field (Poissenot et al.). The great value of long-term field studies cannot be overestimated: 34 years for a badger population ! (Allen et al.). *Behaviors* reported range from the bizarre "traumatic mating" of hyloid frogs whose males scratch the skin of females to inject pheromones (L. Schulte and Bossuyt) to new understanding of enigmatic behaviors such as the cats' nepetalactone rolling and rubbing (Miyazaki and Uenoyama; and Uenoyama et al.)

***Practical Applications.*** The holy grail for many researchers has long been the search for effective applications, particularly in *detecting health disorders by animal noses*. Mice can be trained to detect changed volatile spectrum in melanoma mice before clinical symptoms occur (Kokocińska-Kodiak and Jezierski). Female mice can detect the lower levels of brevicomine and thiazoline at early stages of cancer in male mice, but with no effect on those females' mate choice (Gouzerh et al.). Virus-infected male mice reduce their MUP production (Hurst and Beynon). Jezierski and Dzięcioł critically review what we know about dogs' ability to detect cancer odors in *humans*. Dogs can be trained to respond to volatiles in breath to detect PTSD panic attacks early (Dalhousie group). In the search for an epileptic seizure odor, dogs were able to predict seizures over 4 hrs in advance (Catala et al.). The chemical senses can play a pivotal role in *Animal Conservation*. Parts of that story are elephant repellents (B. Schulte et al.) and the European mink's antipredator responses in connection with reintroduction projects (Ortiz-Jimenez and Barja). Finally, *Animal Welfare* benefits as when artificially fed lambs gain weight faster when ersatz nipples are scented with inguinal wax of maternal sheep (Alary et al.)

It would be presumptuous to try to predict the future, but some growing points offer themselves: We will ever better understand the subtleties and complexities of the chemical language of animals, embedded with other modalities. We will drill deeper into the biosynthesis of semiochemicals and the genetic underpinnings of behavior and chemoreception. The brain will reveal more of its workings. Portable, miniaturized and more sensitive equipment will open up more uses, particularly in the field. Reflecting all that complexity, research teams will grow ever larger. And what will be the future of virtual meetings? As for this one, I wish all a stimulating and productive exchange of ideas!

# Organizing Team

## Organizers

Benoist SCHAAL (CNRS, Dijon, France)  
Matthieu KELLER (CNRS, Nouzilly-Tours, France)

## Local Organizing Committee

Diane REKOW (event coordinator, website administrator & GatherTown coach, communication)  
Vincent GIGOT (on-site tech support, video recording)  
Stéphanie BUSSET (website, registration)  
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Benjamin NOURRY (on-site tech support, informatics)  
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Hélène LOOS (Universität Erlangen-Nürnberg & Fraunhofer Institute, Germany)  
Craig ROBERTS (University of Stirling, UK)  
Tristram WYATT (Oxford University, UK)

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- Université de Bourgogne-Franche-Comté
- Conseil Régional de Bourgogne-Franche-Comté
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- Equipe de Neuroendocrinologie des Interactions et Comportements Sexuels, Laboratoire de Physiologie de la Reproduction et des Comportements, UMR 7247, Nouzilly.
- Département de Physiologie Animale et Systèmes d’Elevage, Inrae
- Département Alimentation Humaine, Inrae
- Groupement de Recherche ‘Médiatéc’ du CNRS
- Groupement de Recherche ‘Odorant, Odeur, Olfaction’ du CNRS
- Société de Neuroendocrinologie
- European Chemoreception Research Organization



# Wednesday 3 Nov

9:00-9:15: *OPENING SESSION FOR CSIV 15 : Welcome and various information*

9:15-10:00: **PLENARY** Bettina Pause Chair: Benoist Schaal  
Smelling the basis of social connectedness: Chemosensory communication in humans

10:00-12:15 **THEMATIC SESSION** Chair: Camille Ferdenzi  
*Human chemical communication*

10:00-10:05 Introduction: C Ferdenzi

10:06-10:24 Body odors: methods to elucidate their chemical composition and determine diet-related influences. H Loos

10:25-10:43 Does handshaking provide a means of olfactory communication in humans? SC Roberts, F Singleton & J Havlíček

10:44-11:02 Human communication of sickness cues. M Olsson

11:02-11:20 Break

11:20-11:38 Chemosignals: a tool for wellbeing. V Parma

11:39-11:57 Body odour cues to men's physical fitness. J Třebická-Fialová, V Třebický & J Havlíček

11:58-12:16 Does human body odor contain cues to immunoreactivity? D Schwambergová, A Sorokowska, Ž Slámová, J Třebická-Fialová, A Sabiniewicz, J Nowak-Kornick, B Borkowska, B Pawłowski & J Havlíček



## 12:16 - 13:15 Poster Session (daily suggested selection)

- 01 - Impact of diets unbalanced in n-3 polyunsaturated fatty acids on the mouse olfactory function. V Soubeyre, L Merle, D Jarriault, S Grégoire, L Bretillon, N Acar, X Grosmaître & AM Le Bon.
- 02 - Detection of buck olfactory pheromones by goats: a calcium imaging approach. M Meunier, C Porte, D Chesneau, AC Trouillet, C Le Danvic, P Nagnan Le Meillour, P Chemineau, P Chamero & M Keller
- 03 - Evaluation of feline semiochemical interactions with Cat vomeronasal type-1 receptor members using the multiple-ligand simultaneous docking (MLSD) approach. R Durairaj, C Bienboire-Frosini, A Cozzi, P Pageat
- 04 - Sex differences in mice exploratory behaviour to Fel D 1, a cat ABP-like protein. C Grau, C Bienboire-Frosini, S Arroub, C Lafont-Lecuele, J Leclercq, P Pageat
- 05 - Sensory detection by Gai2+ VSNs modulates experience-dependent social behaviors in female mice. AC Trouillet, C Moussu, K Poissenot, M Keller, L Birnbaumer, T Leinders-Zufall, F Zufall, P Chamero.
- 06 - Painting inguinal wax on artificial nipples enhances weight gain in artificially fed lambs. J Alary, B Schaal, B Patris, G Chotro, A Destrez

## 13:15- 14:17 THEMATIC SESSION II

Chairs: Helene Loos & Benoist Schaal

### *Chemoreception in neonatal organisms: Signals for survival*

13:15-13:20 Introduction: H Loos

13:21-13:39 Evaluation of tadpole alarm substance extracts on behavioural anti-predator response in three anuran species. K Lipkowski, D Abondano, D Wenzel & L Schulte

13:40-13:58 Does perinatal chemo-variety experience shape adult personality? Exposure to chemosensory variety *in utero* or *in lacto* and emotional responsiveness to novelty in adult mice. B Patris, A Dastugue & B Schaal

13:59-14:17 A non-mammary source for the rabbit mammary pheromone: Multiple vital functions for a same chemosignal. AS Moncomble, D Langlois & B Schaal

## 14:20- 15:10 KEYNOTE

Brian Dias

Chair: Christine Drea

*Using olfaction to understand intergenerational influences of parental sensory experiences.*

15:10 – 15:25 Break

15:25-16:40 OPEN SESSION 1

Chair : Christina Buesching

*Intraspecific odor communication : Sources, chemosignals, and functions*

15:25-15:43 Characterization of the olfactory communication in water voles, *Arvicola terrestris*. K Poissenot, AC Trouillet, C Moussu, D Chesneau, V Lattard, J Drevet, C Le Danvic, P Nagnan-Le Meillour, P Chamero & M Keller

15:44-16:02 Odor-based MHC recognition in kittiwakes. S Leclaire, P Blanchard, M Pineaux

16:03-16:21 Scent-marking with metabolic by-products versus specialised glandular secretions: a case study with European badgers (*Meles meles*). T Allen, C Newman, D Macdonald, & C Buesching

16:22-16:40 Competitive Scent Signalling in Female Voles. H Coombes, G Loxley, R Beynon & JL Hurst

Closing day 1 of meeting

# Day 1 Abstracts

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## Plenary

### Smelling the basis of social connectedness: Chemosensory communication in humans

Bettina M. Pause

Heinrich-Heine-University, Düsseldorf, Germany

Humans convey numerous information through social volatiles, e.g., about their genetic profile, sex, age, nutrition and health status, personality characteristics and emotions.

Concerning emotional communication, the impact of chemosensory signals varies with sender and perceiver characteristics: Depending on the type of emotion, men and women respond differently to the respective chemosensory signals. Chemosensory stress signals seem to be processed in men and women alike, however, chemosensory signals of fear and anxiety seem to predominantly affect women and not men. Similarly, chemosensory signals of male aggression are processed more strongly in the female than the male brain. Furthermore, the impact of a specific chemosensorily transmitted emotion depends on the perceiver's neuronal and hormonal status. While the processing of chemosensory anxiety signals is augmented in socially anxious individuals, it is attenuated in pregnant women. Intriguingly, multisensory studies show that in perceptually ambiguous situations, chemosensory information overrides social information transmitted through other senses. Moreover, chemosensory communication most often acts below the attentional threshold, strongly reducing the perceivers' capacity to intentionally regulate its emotional impact.

In sum, by means of chemosignals, humans are able to differentiate between safe versus dangerous social connections. Ontogenetically and phylogenetically harmful conditions like social stress and aggression, illness or hunger are avoided. First studies indicate that social skills, reflected in the social network size or in the level of empathy, are directly linked to olfactory skills. Even the selection of friends seems to be based on the allelic profile of olfactory receptor expression.

It is concluded that in humans, chemosensory messages might represent honest signals, thus forming the most valid predictors of the consequences of social encounters. It will be discussed whether the sense of smell aided in the formation of the social brain by forming the prerequisite for social connectedness.

# Thematic Session I

## Chemical communication of humans' physiological and psychological states

Chair: Camille FERDENZI

Centre de Recherche en Neurosciences de Lyon CNRS UMR 5292, Lyon, France

Olfactory communication exists in many species, including humans. The quest for human pheromones has motivated many studies in this area, with disputable outcomes. In recent years, new approaches favor the understanding of a communication system where complex odors produced by the body could convey adaptive odor cues or signals. We are now trying to understand how chemosignaling contributes to our health, well-being, bonding and mating. This symposium aims at providing some answers to the question how information about an individual's physiological and psychological state can be conveyed through body odor. Specifically, human body odor is likely to contain cues of emotional state, sickness, nutritional state, which could cause adaptive behavioral modifications in the receivers.

Speaker #1 Helene LOOS

Fraunhofer-Institut für Verfahrenstechnik und Verpackung IVV, Freising, and Friedrich-Alexander-Universität Erlangen-Nürnberg, Erlangen, Germany

***Body odors: methods to elucidate their chemical composition and determine diet-related influences***

Speaker #2 Craig ROBERTS

University of Stirling, Scotland, UK

***Does handshaking provide a means of olfactory communication in humans?***

Speaker #3 Mats OLSSON

Karolinska Institutet, Stockholm, Sweden

***Human communication of sickness cues***

Speaker #4 Valentina PARMA

Monell Chemical Senses Center, Philadelphia, USA

***Chemosignals: a tool for wellbeing***

Speaker #5 Dagmar SCHWAMBERGOVA

Charles University, Prague, Czech Republic

***Does human body odor contain cues to immunoreactivity?***

Speaker #6 Jitka TŘEBICKÁ FIALOVÁ

Faculty of Science, Charles University, Prague, Czech Republic

***Body odour cues to men's physical fitness***

## Body odors: methods to elucidate their chemical composition and determine diet-related influences

Helene Loos

Department of Chemistry and Pharmacy, Friedrich-Alexander Universität Erlangen-Nürnberg, Henkestraße 9, 91054 Erlangen, Germany, and Department of Sensory Analytics & Technologies, Fraunhofer Institute for Process Engineering and Packaging IVV, Giggenhauser Straße 35, 85354 Freising, Germany

Volatiles and/or odour-active substances emitted from different sites and fluids of the body constitute the human volatilome. Being largely influenced by internal physiological processes, it has since long been recognized as a means to monitor human health. In more recent decades, however, research on human chemical communication led to evidence that besides the health status, other psychophysiological conditions are also reflected in parts of the human volatilome. To elucidate the nature of molecules associated with specific states or traits of the sender, suitable methods for the sampling and the analysis of the emitted volatiles are needed along with proper data analysis. Currently, mass spectrometric methods in conjunction or not with gas chromatography are commonly used for the analysis of the human volatilome. In addition, olfactometry can be applied to specifically target odour-active trace compounds. Besides being influenced by internal physiological processes, the composition of the human volatilome is also dependent on environmental conditions, on personal hygiene, and on the intentional or unintentional uptake of substances from the environment, from food, medication or similar, or from products of daily use that are applied on the body. Such external influences can either directly or indirectly affect the volatilome and also bodily odours. Together with a discussion of different methods used for the sampling and the analysis of the human volatilome, an overview of recent insights into the alteration of the odours and/or the composition of bodily fluids by dietary intake of aroma-active substances will be given, with a specific focus on human milk, urine, and breath.

## Does handshaking provide a means of olfactory communication in humans?

S. Craig Roberts (1), Francesca Singleton (1) & Jan Havlíček (2)

(1) Division of Psychology, University of Stirling, Scotland, UK

(2) Dept of Zoology, Charles University, Czech Republic

In many species, meetings between individuals are characterised by brief or even extensive olfactory investigation. Inter-individual assessments made in this way enable individual discrimination or recognition and perhaps shape appropriate responses in subsequent interaction, such as in decisions about escalating aggression or whether to accept a mate. A recent study claimed that in humans, handshake greetings might have a similar role: it suggested that people often smell their hands after a handshake. I will present results of a study aiming to further test this idea.

Experimental participants attended a session in which they greeted each of four confederates in a staged interaction lasting 90 seconds. Approximately 180 participants were tasked with remembering information about the confederates. Interactions were scripted for confederates, in order to standardise the task, but at the beginning of the greeting one confederate of each sex shook hands with the participant, the other two confederates did not. This enabled us to compare behaviour of male and female participants to both male and female confederates in a balanced design, and by covertly filming each interaction, we were able to score the extent to which participants assessed the smell of their hands immediately following the greeting. Furthermore, following the interactions, participants underwent a memory task in which they recalled details about the confederates. The results of this study will be presented and the implications of the results discussed, as well as comments on the ethical and procedural difficulties presented by such studies.

## Human communication of sickness cues

Mats Olsson

Karolinska Institutet, Department of Clinical Neuroscience, Stockholm, Sweden

Infectious diseases have been a major threat to humans throughout evolution. Accordingly, people tend to avoid other people when they express obvious signals of sickness (coughing, sneezing). Such behavioral defense against disease probably serves us well. Using an experimental endotoxemia model we study the “early” and “subtle” cues of sickness that emerge as individuals develop a systemic inflammation. We here review these studies targeting chemical (skin and urine odor), visual (facial, body motion) and vocal cues. Results indicate that already within hours of the induction of systemic inflammation human participants can detect a number of subtle sickness cues which are likely to promote avoidance behavior when that is appropriate. We can also show how these disease cues can be integrated for a better discrimination between healthy and sick people.

## Chemosignals: a tool for wellbeing

Valentina Parma

Monell Chemical Senses Center, Philadelphia, PA, USA

Social relationships significantly affect wellbeing. The role that a functional sense of smell plays in establishing and maintaining social relationships is under researched, though it is now in the spotlight as a result of the millions of individuals affected by smell loss when only considering COVID-19. In this talk, I will review evidence on the role of chemosignals in fostering healthy relationships over the lifespan, with particular attention to how chemosignals affect emotional decoding and regulation.

Methods: Systematic review with metanalytic components, of published and unpublished findings.

Results: Despite the methodological heterogeneity of the existing data pool, emotional decoding and regulation based on human chemosignals indicates the statistical robustness of the human capacity to using olfactory cues to foster relationships.

Conclusions: A functional sense of smell – including the ability to process human chemosignals - affects the ability to form and maintain healthy relationships.



## Does human body odour contain cues to immunoreactivity?

Dagmar Schwambergová (1), Agnieszka Sorokowska (2,4), Žaneta Slámová (1), Jitka Třebická Fialová (1), Agnieszka Sabiniewicz (2,4), Judyta Nowak-Kornicka (3), Barbara Borkowska (3), Bogusław Pawłowski (3), Jan Havlíček (1)

(1) Faculty of Science, Charles University, Prague - Czech Republic

(2) Faculty of Historical and Pedagogical Sciences, University of Wrocław, Wrocław, Poland

(3) Faculty of Biological Sciences, University of Wrocław, Wrocław, Poland

(4) Smell and Taste Clinic, Technische Universität Dresden, Germany

It has been previously shown that in various mammalian species, body odour might provide cues to health status. However, it may also serve as a cue to some aspects of genetic quality, such as immunoreactivity. Previous studies have shown that women perceive male faces with a more reactive immune system as more attractive, but whether body odour might likewise provide cues to immune function has not been investigated yet. In two studies, we aimed to test a possible relationship between body odour quality and immunoreactivity/health status (Study 1) and immune system functioning (Study 2).

In Study 1, we collected body odour samples from 21 men twice - at the baseline before and two weeks after vaccination against hepatitis A/B and meningococcus. We also obtained participant's blood samples to determine levels of specific antibodies (selected as markers of reactivity of the immune system), testosterone and cortisol. Subsequently, a panel of 88 female raters assessed the body odour samples for their attractiveness, intensity, and healthiness.

In Study 2, we collected body odour and blood samples from 35 men and women. We assessed key parameters of their immune system (e.g., complement activity) and asked 95 raters to assess the samples for their attractiveness, intensity, and healthiness.

In Study 1, we found no significant association between antibody levels induced by vaccination and perceived body odour quality. We also found no significant relationship between antibody levels and steroid hormones. Moreover, contrary to our expectations we found a significant positive change in body odour ratings after vaccination. In Study 2, we also found no association between basal key parameters of the immune system and body odour quality. Our results indicate that body odour may not serve as a cue to the immune system functioning.

Funding: The study was supported by the Charles University Grant Agency (GAUK1398218); Czech Science Foundation (GAČR21-29772S); Owen F Aldis Scholarship, and National Science Centre, Poland (OPUS42012/07/B/NZ8/02666)

## Body odour cues to men's physical fitness

Jitka Třebická Fialová (1), Vít Třebický (1,2) & Jan Havlíček (1)

(1) Faculty of Science, Charles University, Prague, Czech Republic

(2) Faculty of Physical Education and Sport, Charles University, Prague, Czech Republic

People tend to make relatively accurate judgments about men's physical performance (e.g., strength) or fighting ability based on visual and vocal cues. Similarly, body odour quality has been shown to be associated with characteristics related to an individual's qualities such as symmetry or health status. However, it is not clear whether human body odour can provide cues to physical fitness. Here, we investigated the possible relationship between perceived body odour quality and selected aspects of physical performance in Czech Mixed Martial Arts (MMA) fighters. We collected body odour samples from 44 MMA fighters (mean age: 26.6 years, SD 5.9 years) (sampled for 30 minutes onto cotton T-shirts). Further, we obtained data regarding their physical (e.g., age, height, body composition) and performance (MMA score, muscle strength, lung capacity, anaerobic performance) characteristics. Obtained odour stimuli were subsequently rated on a 7-point scale regarding their pleasantness, attractiveness, and intensity by 65 women (mean age: 23 years, SD 4.7 years). Results from the linear mixed effect models showed that none of the physical or performance characteristics predicted perceived body odour quality. Interestingly, we found a positive association between the intensity of body odour and the MMA score. Our findings thus suggest that body odour can provide cues to only certain aspects of men's physical fitness.

Funding: Czech Science Foundation (grant no. 21-29772S)

# Thematic Session II

## Chemoreception in neonate organisms: Signals for survival

Chairs: Helene Loos (1) & Benoist Schaal (2)

(1) Friedrich-Alexander University of Erlangen-Nuremberg and Fraunhofer Institute for Process Engineering and Packaging IVV

(2) CNRS and Université de Bourgogne

Larval, newly-hatched or newly-born vertebrates must optimally manage the precarious conditions of their transitional natural history. To survive early vicissitudes, they must express strategies allowing them to escape predators or parasites, find sheltering environments and adequate nutrients, and, in the rare taxonomic cases where they benefit from parental precaution, respond timely to the multiple gains distributed by parents. To fulfill all these constraints, vertebrate 'newborns' have evolved multiple, non-exclusive strategies. They may come to the world with sensory biases that orient behavioral automatisms toward evolutionarily expected or avoided stimuli. In parallel, they are in a state of maximal plasticity of brain and behavior, allowing the rapid and stable learning of the local specificities of their social and biotic environment.

This symposium will illustrate three instances of adaptive sensing and responding in immature 'neonates'. A first talk will be on how anuran tadpoles are alarmed about predation risk via chemicals released by homo- or heterospecific tadpoles subject to tissue damage. A second talk will present an assessment of the long-term impact on 'personality' of being exposed to ambient chemosensory variety during perinatal life in the laboratory mouse. Finally, the European rabbit complexifies its case in emitting a mammary pheromone not only in milk, but also from non-mammary sites, fulfilling multiple functions to boost the survival and successful thriving of neonates.

Speaker #1 Konrad Lipkowski

Department of Wildlife-/Zoo-Animal-Biology and Systematics, Institute for Ecology, Evolution and Diversity, Goethe University Frankfurt, Germany

***Evaluation of tadpole alarm substance extracts on behavioural anti-predator response in three anuran species***

Speaker #2 Bruno Patris

Centre for Taste, Smell and Feeding Behavior, CNRS, Université de Bourgogne, Dijon, France

***Does perinatal experience with chemosensory variety shape adult personality? Exposure to flavor variety in utero or in lacto and later emotional responsiveness to novelty in mice***

Speaker #3 Benoist Schaal

Centre for Taste, Smell and Feeding Behavior, CNRS, Université de Bourgogne, Dijon, France

***A non-mammary source for the rabbit mammary pheromone: Multiple vital functions for the same chemosignal***

## Evaluation of tadpole alarm substance extracts on behavioural anti-predator response in three anuran species

Konrad Lipkowski\*, Diana Abondano, David Wenzel & Lisa Schulte

Department of Wildlife-/Zoo-Animal-Biology and Systematics, Institute for Ecology, Evolution and Diversity, Goethe University Frankfurt, Max-von-Laue-Straße 13, 60438 Frankfurt am Main, Germany.

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Tadpoles can assess the ambient predation-risk through the perception of chemical cues associated with predation (alarm substances) and adjust their behavioural activities accordingly. This includes acute behavioural changes caused by chemicals solely released by injured conspecifics (damage-released-alarm-substances; DRAS). Although our understanding of predator-prey interactions mediated by alarm substances in tadpoles is steadily increasing, studies exploring the chemical aspects of DRAS remain scarce and at times contradictory. Furthermore, preparation methods of DRAS for subsequent trials differ between studies, making it at times difficult to disentangle an influence of extraction methods and context in which anti-predator mechanisms are explored. To investigate this and help facilitate our understanding of chemical aspects of alarm substances, we conducted a range of behavioural trials with differently extracted and manipulated DRAS (including heterospecific, flash freezing and extracts using Tricain) across three phylogenetic distinct anuran species. Tadpoles of *Bufo bufo* (Bufonidae), *Rana temporaria* (Ranidae) and *Ranitomeya sirensis* (Dendrobatidae) were assigned to one of 30 treatments and recorded for subsequent comparison of corresponding behaviour before and after stimulus application. Our experiments aimed to draw conclusions about chemical elements including phylogenetic conserved and/or convergent elements as well as potential impact of differently extracted DRAS for the interpretation of behavioural-ecological results.

## Does perinatal experience with chemosensory variety shape adult personality? Exposure to flavor variety in utero or in lacto and later emotional responsiveness to novelty in mice

Bruno Patris, Aurélie Dastugue, & Benoist Schaal

Developmental Ethology and Cognitive Psychology Group; Centre for Taste, Smell and Feeding Behavior, CNRS, Université de Bourgogne, Dijon, France

The chemicals that mammalian females ingest, inhale or contact during pregnancy or lactation permeate more or less easily and rapidly to their fetal or neonatal offspring. These compounds often convey chemosensory properties and therefore impinge on the offspring's olfaction, taste or chemesthesis, shaping their subsequent chemosensory functioning and affecting their preferences and behavior into adulthood. Such long-term postnatal effects of prenatal experience have been demonstrated after exposure to pure flavorants or single flavor qualities, which induce selective responses postnatally. Here, we aim to examine whether and how perinatal exposure to multiple, varied flavors can influence later non-selective responsiveness to novelty. Specifically, we compared the behavior of adult mice born to pregnant or lactating dams fed a regimen of flavor variety vs. a regimen of flavor monotony, in terms of expressed level of stress or avoidance to novelty. Perinatal exposure to chemo-variety was expected to decrease later stress level in a novel environment and neophobic responsiveness toward unfamiliar chemostimuli. Chemo-variety exposure was achieved by odorizing gestating/lactating dams' drinking water with accepted solutions of six distinct flavorants, changing each day, over a period of 6 days before or 6 days after birth. The monotonous treatment was achieved by odorizing gestating/lactating dams' drinking water with only one of the six flavorants. After weaning, the mice were subjected to three behavioral tests measuring their reactivity to novel environments or stimuli; i) at day 26, an exploration test in a T-maze assessed reactions to a novel vs. familiar odorant; ii) at day 29, an exploration test in an open-field measured emotional reactions in presence or not of a non-familiar object; and iii) at day 33, an ingestion test evaluated differential intake of newly-flavored water vs. scentless control water. Both exploration tests indicated positive impacts of early chemo-variety exposure on stress and neophobic behavior, mice pre- or postnatally exposed to chemo-variety exploring more potentially stressful areas. However, the ingestion test appeared inconclusive. Significant sex differences were also noted. Females displayed higher activity levels in the T-maze and Open-field tests and seemed more positively impacted by the perinatal chemosensory enrichment.

## A non-mammary source for the rabbit mammary pheromone: Multiple vital functions for the same chemosignal

Anne-Sophie Moncomble, Dominique Langlois & Benoist Schaal

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The odorant 2-methyl-but-2-enal (2MB2-al) controls interactions between rabbit (*Oryctolagus cuniculus*) females and their newborn pups. It is released into milk (accordingly coined 'mammary pheromone') wherein it elicits pups' vigorous searching, oral grasping and sucking. Pups' initial reactivity to it is prognostic to their long-term viability. A second function of 2MB2-al is the engagement of learning of any contingent odorant, which becomes then as potent as the pheromone itself to elicit searching-grasping. Here, we describe a third function of this chemosignal that consists in the attraction/appetence of pups to maternal feces.

While suckling, dams variably release a small amount (2-3/day) of hard fecal pellets (FP) into the nest up to days 10-12 postpartum (Hudson 1996; Moncomble et al 2004; Kovacs et al 2006). Pups trace these FP in the nest material and fragment them by a typical foreleg scratching behavior, before ingesting them progressively. Follow-up of the pups' behavior toward FP in the nest indicates that their ingestion is effective from postnatal days 2-3. Two-choice preference tests exposing pups to various kinds of FP found attraction regardless of the physiological state of the emitting female. When exposed to FP before and after excretion, pups were more attracted to excreted FP, which are coated with secretion from anal glands (AG). Dynamic headspace extraction (on Tenax) and subsequent GC-MS analyses revealed different volatile profiles of excreted vs. non-excreted FP. In particular, traces of 2MB2-al and its alcohol, 2MB2-ol, were detected in the effluvium of excreted PF but not of non-excreted PF. Further, the headspace of AG from latent females contained both 2MB2-al and 2MB2-ol. Thus, during anal transit, hard FP are charged with 2MB2-al and 2MB2-ol emitted from AG. Subsequent olfactory testing showed that synthetic 2MB2-ol was behaviorally inactive on pups, suggesting that attraction to FP is prevalingly due to 2MB2-al.

The process of 2MB2-al production in AG remains unknown, but this and earlier studies (Hesterman & Mykytowycz 1968) point it as a regular component of adult rabbit FP effluvium. In the nest context, 2MB2-al may be causal in initiating attraction/ingestion of maternal FP by pups, exposing them at once to the flavors of the mother's safe diet and to non-pathogenic microbiota that rapidly colonize their oro-digestive tract. Pups' intake of maternal FP do effectively speed up implantation of mature caecal microbiota and reduce mortality (Combes et al 2014). In sum, in addition to its releasing effects on milk intake, 2MB2-al favors contact with non-milk items (FP) i) carrying odor cues of foodstuffs preselected by the lactating mother and ii) replete with microbiota capable of digesting them. Thus, the mammary pheromone emitted from multiple sources engages multiple vital behavioral and physiological adaptations in newborn rabbits.

## Keynote

# Using olfaction to understand intergenerational influences of parental sensory experiences

Brian Dias

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Division of Research on Children, Youth & Families, Children's Hospital Los Angeles, USA

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Intergenerational influences of salient parental environments have the potential to either shape adaptations or constrain biology in offspring. For example, exposure of parental populations to salient environmental stimuli like predation, sensory experience, and endocrine disruptors exert strong influences on physiology and behavior of offspring. Despite such far-reaching impact, there are gaps in our knowledge about the mechanisms via which information about salient parental (G0) environments is passed to offspring (G1), and the ensuing consequence(s) for the G1 generation. To fill these gaps, we leverage the accessibility and organizational principles of the olfactory system. Doing so allows us to follow structural (visualize olfactory neurons), functional (behavior after detecting specific odors) and genetic (examine loci encoding specific odorant receptors) influences of G0 experience, across generations. My talk will highlight this approach and leave room for discussion about whether such an approach could address concepts like the evolution of instinct.

# Open Session 1

## Intraspecific odor communication : Sources, chemosignals, and functions

Open Session 1-1

### Characterization of the olfactory communication in water voles, *Arvicola terrestris*

Kévin Poissenot (1), Anne-Charlotte Trouillet (1), Chantal Moussu (1), Didier Chesneau (1), Virginie Lattard (2), Joël Drevet (3), Chrystelle Le Danvic (4), Patricia Nagnan-Le Meillour (5), Pablo Chamero (1) & Matthieu Keller (1)

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In mammals, notably in rodents, social behaviors like attraction of sex partner, territoriality, or parenting, largely rely on olfactory communication by pheromones. There are species-specific chemosignals, including small volatiles, sex steroids, or proteins secreted in the urine or by various exocrine glands. The detection of pheromones is mainly ensured by the olfactory neurons of two specific sensory organs, the vomeronasal organ (VNO) and the main olfactory epithelium (MOE). This study aims to characterize the olfactory communication in the fossorial ecotype of water voles, *Arvicola terrestris*. This vole of western Europe colonizes permanent meadows in mid-mountains where it digs an extensive gallery network to live in couple with its progeny. Previously, we explored the chemical ecology of water voles. Specific volatile compounds differ between males and females and could therefore serve as an attractant for conspecifics. First, we evaluated the attractiveness of urine and lateral scent gland secretions to voles. Voles were simultaneously exposed to secretions from males and females in a two-choice olfactory test in an animal facility and in the field. Our results show an attractiveness of urinary male odor both in the laboratory and in the field. Second, we demonstrated the ability of VNO and MOE olfactory neurons to detect volatile compounds found in water vole secretions by *in vitro* calcium imaging. Finally, we evaluated the use of two mixtures of volatiles from urine or lateral scent glands to enhance trapping of the vole in fields undergoing a cyclic outbreak in vole demographics.



## Odor-based MHC recognition in kittiwakes

Sarah Leclaire, Pierrick Blanchard & Maxime Pineaux

Laboratoire Evolution & Diversité Biologique, Toulouse, France

In a wide range of taxa, individuals use odor cues during mate choice to assess the genetic characteristics of potential partners. However, in contrast to fish, lizards or mammals, the ability of birds to assess genetic traits using olfactory cues has long been unexplored. Here, we focused on genes of the Major Histocompatibility Complex (MHC), which are essential for resistance to pathogens and thus fitness in vertebrates. We studied the existence of odor-based MHC recognition and its potential ultimate and proximate mechanisms (i.e., why it is evolutionary adaptive and how the odor cue is produced) in a seabird species, the black-legged kittiwake. First, using behavioral tests, we provided evidence that female kittiwakes can assess male's MHC characteristics using odor cues. Then, we found that MHC-diversity predicts fitness in kittiwake chicks and adults. MHC odor-based recognition may thus be involved in behavioral strategies that increase chicks' MHC diversity, such as MHC-based mate choice. Third, we started investigating whether MHC influences odor indirectly by shaping the community of odor-producing bacteria hosted on feathers. We found that the composition and diversity of feather and cloacal microbiota were related to fitness in kittiwakes. Studies are ongoing to determine whether these relationships may be driven by MHC-related fitness traits.

## Scent-marking with metabolic by-products versus specialised glandular secretions: a case study with European badgers (*Meles meles*)

Tanesha Allen, Chris Newman, David Macdonald, & Christina Buesching

Wildlife Conservation Research Unit, Department of Zoology, Reanati-Kaplan Centre, University of Oxford, Tubney, Abingdon, Oxfordshire, UK

Scent-marking maintains social cohesion among populations by advertising fitness-related information (e.g., age, sex, reproductive status) about individuals. Conspecifics encountering deposited marks can then assess encoded information and evaluate their fitness relative to that of the scent donor, thus influencing decisions regarding future social interactions. Both metabolic by-products (i.e., urine and faeces) and specialised scent secretions are utilised during scent-marking, and both substances encode similar information. However, few studies have directly compared these marking modalities and the evolutionary advantage of advertising similar information in two different ways. European badgers (*Meles meles*) rely predominantly on olfactory communication due to their nocturnal and fossorial lifestyles. These highly macromammalian mustelids scent mark their territories, resources, and conspecifics with metabolic by-products and specialised scent secretions from paired anal glands and a uniquely evolved subcaudal gland. Numerous GC-MS studies and scent-provisioning experiments have demonstrated that: i) metabolic by-products and specialised scent secretions encode for individual- and group-related information and ii) badgers residing in wild populations can decipher fitness-related information from scent marks, adjusting their behaviour accordingly. Using data collated since 1987 from a wild badger population in Wytham Woods, we performed a meta-analysis comparing scent-marking with metabolic by-products (i.e., urine) versus specialised glandular secretions (i.e., subcaudal gland secretion). With GC-MS profiles and behavioural observations, we discuss the physiological and social costs and benefits associated with each modality.

## Competitive Scent Signalling in Female Voles

Holly Coombes (1), Grace Loxley (1), Rob Beynon (2), & Jane Hurst (1)

(1) Mammalian Behaviour & Evolution Group, Institute of Infection, Veterinary & Ecological Sciences, University of Liverpool, Neston, UK

(2) Centre for Proteome Research, University of Liverpool, Liverpool, UK

Intrasexual competition for reproductive opportunities leads to the evolution of secondary sexual signals that indicate competitive ability and individual quality. Factors influencing the evolution of such signals vary between the sexes due to different costs of reproduction for males and females. Chemical signals are widely used for competitive and reproductive signalling across taxa and have been well studied in males. However, signalling among females has been largely overlooked. Here, we investigate female scent signalling in two species of voles with typically overlapping (*Microtus agrestis*) or exclusive (*Myodes glareolus*) home ranges. Adult females were established in home enclosures linked to a neighbour enclosure that housed a familiar littermate or an unfamiliar and unrelated conspecific. Investment in urinary scent marking and protein components of scents was measured before and after controlled interactions between neighbours. Female competitive behaviours and space use were measured as indicators of female competitive strategy. As predicted, female *M. agrestis* invested more in urinary scent signalling than *M. glareolus*, both through overall protein output and deployment of scent. Female *M. agrestis* express high levels of multiple olfactory binding proteins in their scents with no equivalent scent proteins in female *M. glareolus*. Individual female *M. agrestis* with high urinary protein output also showed greater investment in scent marking, a relationship that was lacking in *M. glareolus*. By contrast, female *M. glareolus* tended to spend more time in direct aggression, consistent with different female competitive strategies in the two species. However, individual differences in female scent investment were not directly related to individual differences in competitive behaviour in either species, in contrast to typical signalling among males. Our findings suggest that patterns of female signalling differ between closely related species according to competitive strategies, while factors driving female competitive signalling may differ from those in males.

# Thursday 4 Nov

9.00-9.10: Welcome - information

9.10-10.00: **PLENARY** Jonathan Williams Chair: S Craig Roberts  
*Chemical emissions and signals from human beings*

10:01-12:06 **THEMATIC SESSION III** Chair: Marianne Gabirot  
*Innovative methods in semiochemistry: from sampling to identification, statistical validation and reproducibility*

10:01-10:06 Introduction : M Gabirot

10:07- 10:32 From swab to spectrum: a comprehensive guide to simple GC-MS mammalian scent analysis. E Nonnamaker, M Muszynska, I Lightcap, CM Drea & EA Archie

10:33-10:58 Finding the "needle in the haystack": a comparison of statistical tools for untargeted chemical analysis. BM Weiß, E Kusch, M Kücklich, A Widdig

11:00-11:15 **Break**

11:15-11:40 Evaluation and contextualisation of volatile organic compounds from breath samples of type 1 diabetes during hypoglycaemia, hyperglycaemia and euglycaemia. Z Parr-Cortes, NJ Rooney, M Wheatstone, T Stock, C Pesterfield, C Guest & CT Müller

11:41-12:06 Life scientists in chemical ecology can benefit from psychology's responses to its own 'reproducibility crisis'. T Wyatt

## 12:10 – 13:10 Poster Session (daily suggested selection)

- 07 - The testosterone-dependent felinine production is upregulated by an inhibition of the cholesterol biosynthesis in mice. N Doi, S Ichizawa, R Uenoyama, & M Miyazaki
- 08 - The lip-smacking response of male dogs toward vaginal secretion and urine of estrous female dogs. T Miyazaki, U Onozawa, R Uenoyama, & M Miyazaki
- 09 - Licking and chewing silver vine leaves by domestic cats enhances their ability to gain iridoids for chemical defense against pest insects. R Uenoyama, T Miyazaki, JL Hurst, M Adachi, T Nishikawa, & M Miyazaki
- 10 - Structural brain plasticity and olfactory function in a mouse model of congenital blindness. N Bouguiyou, G Bronchti, D Galino, MM Chakravarty, JA Frasnelli & S Al Ain
- 11 - Comparative anatomy of the olfactory system of Neotropical poison frogs and its coherence with chemical communication. M Schreier, D Abondano Almeida & L Schulte
- 12 - Signal or cue – search for clues about the release mechanism of damage-released alarm substances. K Lipkowski, D Wenzel & L Schulte

**13:10-13:50 KEYNOTE**                      **Fabrice Neiers**                      **Chair: Rob Beynon**  
**Enzymatic systems involved in peri-receptor events of olfaction in Mammalia: evolutionary perspectives**

**13:51-15:12 THEMATIC SESSION IV**                      **Chairs: Arnaud Leleu & Isabelle Charrier**  
***Chemical signals in a multisensory environment***

13:51-13:56 Introduction: A Leleu

13:57-14:15 Insight into the sensory ecology of raptors: Vision and olfaction. S Potier, O Duriez, A Célérier, F Bonadonna

14:16-14:34 How odors assist the developing visual system in humans. D Rekow, A Leleu, A Kiseleva, K Durand, B Schaal, B Rossion & JY Baudouin

14:35-14:53 The 'language' of scent within multimodal lemur signals. CM Drea & LK Greene

14:54-15:12 Multimodal recognition of the pup by Australian sea lion mothers. BJ Pitcher, K Wierucka, R Harcourt & I Charrier

15:15-15:30 Break

15:30-16:55 OPEN SESSION 2

Chair: Richard E. Brown

*Olfactory processes: From ecology to cognition, through sex and imprinting*

15:30-15:48: Captivity and Communication: Comparative Analysis of Scents from Wild and Captive Tamarins. A C Poirier, JC Dunn, M Watsa, GA Erkenwick, AD Melin, JS Waterhouse, & AC Smith

15:49-16:17: Mental gland chemical composition and sexual variation in the Spanish terrapin (*Mauremys leprosa*) and preliminary comparison with other chelonian species. A Ibáñez, A Martínez-Silvestre, D Podkowa, A Woźniakiewicz, M Woźniakiewicz & M Pabijan

16:18-16:36 Father-partner body odour similarity: Perceptual, microbial, and chemical dimensions. J Havlicek, L Kuncová, R Hanus, J Kreisinger, P Kyjaková, R Bušovská, L Schmiedová, J Třebická Fialová, D Schwambergová, K Šípková, & Z Štěrbová

16:37-16:55 Short, long and very long-term olfactory memory in mice. RE Brown & OK Schnare & K Roddick

17:00-17:50 KEYNOTE

Lisa Schulte

Chair: Tristram Wyatt

*Traumatic mating in amphibians: male frogs (Plectrohyla, Hylidae) use their teeth to inject females with SPF-pheromones.*

Closing day 2 of meeting

# Day 2 Abstracts

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## Plenary

### Chemical emissions and signals from human beings

Jonathan Williams

Max Planck Institute for Chemistry, Mainz, Germany

Human beings emit a wide variety of trace gases into the air around them. These chemicals can be emitted directly on our breath and from our skin, or generated indirectly through reaction with oxidants such as ozone. These continuous chemical broadcasts involve several hundred volatile organic compounds at ppbv to pptv levels that can be measured with online and offline mass spectrometric methods such as PTR-ToF-MS and GC-MS. In this presentation, it will be shown how a cinema can be used to assess real world human chemical emissions and even find trace gas signals related to the audience's emotional state. These signals have been exploited to determine the age classification of films. Afterwards a climate-chamber based laboratory assessment of human emissions under controlled conditions was used to assess how these human emissions varied as a function of temperature, humidity, ozone, age and clothing coverage. OH reactivity measurements were used to confirm that the majority of reactive VOC was measured, and human ammonia measurements were found to be very sensitive to temperature. Human exposure to ozone generated carbonyl products that depended critically on humidity, and generated large quantities of fine respirable particles (1-4 nm). Interestingly when human beings encounter ozone, either indoors or outdoors, an oxidation field of OH radicals is generated. The results therefore show that people are a potent mobile source of chemical compounds, particles and even radicals. These findings are relevant to indoor air quality, human chemical signaling and perhaps even to our sense of smell.

## Thematic Session III

# Innovative methods in semiochemistry: from sampling to identification, statistical validation and reproducibility

### Chair: Marianne Gabriot

Laboratoire des Sciences des Risques, Platform on Air Quality Management, IMT Mines Alès, Alès, France

### Symposium outline

Our understanding of chemosensory processes underlying animal behavior and communication is currently limited by several major obstacles. On one side, the volatile organic compounds (VOCs) involved in communication are released in generally low concentrations and in noisy backgrounds, and, on the other side, the detection thresholds of usual chemical analyses are not low enough. Also, molecules emitted by animals are highly variable along polarity, solubility, volatility, and other properties. This leads to a difficult situation to choose the most adapted methods for sampling and chemical analyses. For example, the pre-concentration step used in classical VOCs analysis actually generates biases that are often unavoidable in the analysis of gas samples. Indeed, monitoring of dynamic emission and degradation of VOCs remains complicated with conventional techniques, and this challenge requires innovative analytical methods and equipment. In fact, the capture, extraction, storage, analysis and interpretation of the compounds that act as chemical cues or signals between individuals are yet in an exploratory phase, and there is a crucial and critical need to further adapt studied models, their ecology and the selection of analytic methods/instruments.

The four presentations that follow will provide new information on the improvement of methods from sampling and storage to the statistical analysis of chemical data and their interpretation. In particular, the use of cotton swabs and their GC-MS analysis will be described in primates. In addition, statistical analysis of complex data sets will be addressed in comparing methods, with a suggestion to combine efficient tools to assess the relevance of chemical signals and visualize them among real or modelled complex chemical profiles. Finally, beyond the operational aspects of semiochemistry and on a point that should be considered ahead of any study, the issue of reproducibility of studies on vertebrate chemocommunication is addressed in suggesting study pre-registration and open reporting of data sets.



Speaker #1 Emily Nonnamaker  
Department of Biological Sciences, University of Notre Dame, Notre Dame, IN  
46617, USA.

***From swab to spectrum: a comprehensive guide to simple GC-MS mammalian  
scent analysis***

Speaker #2 Brigitte M. Weiß  
Institute of Biology, University of Leipzig, Germany & Research Group "Primate  
Behavioural Ecology", Department of Human Behaviour, Ecology and Culture,  
Max-Planck-Institute for Evolutionary Anthropology, Leipzig, Germany

***Finding the "needle in the haystack": a comparison of statistical tools for  
untargeted chemical analysis***

Speaker #3 Zoe Parr-Cortes  
Bristol Veterinary School, Bristol, United Kingdom,

***Evaluation and contextualisation of volatile organic compounds from breath  
samples of type 1 diabetes during hypoglycaemia, hyperglycaemia and  
euglycaemia.***

Speaker #4 Tristram Wyatt  
University of Oxford, Zoology

***Life scientists in chemical ecology can benefit from psychology's responses to its  
own 'reproducibility crisis'***

## From swab to spectrum: a comprehensive guide to simple GC-MS mammalian scent analysis

Emily Nonnamaker (1), Marlena Muszynska (1), Ian Lightcap (2,3,4), Christine M. Drea (5), & Elizabeth A. Archie (1)

(1) Department of Biological Sciences, University of Notre Dame, Notre Dame, IN, USA.

(2) Research and Facilities Program Director

(3) Center for Sustainable Energy, University of Notre Dame, Notre Dame, IN, USA

(4) Materials Characterization Facility, University of Notre Dame, Notre Dame, IN, USA

(5) Department of Evolutionary Anthropology, Duke University, Durham, NC, USA

Advances in the field of analytical chemistry have enabled a rapid acceleration of semiochemical research to understand animal communication. However, mammalian scents remain among the most challenging to identify because of their diverse composition. Here we develop a new workflow for gas chromatography-mass spectrometry (GC-MS) analysis of scents with the aim of generating a robust, feasible, and highly sensitive method for detecting compounds of various volatilities and polarities from mammalian body sites. Here we compare swab materials and their preparation, column types, solventless extraction approaches, and sample queue times using 1. a synthetic mixture of 24 chemical compounds previously identified as potential common chemical cues in a range of vertebrates, 2. genital swabs collected from captive baboons (*Papio anubis*), 3. genital swabs collected from ring-tailed lemurs (*Lemur catta*), and 4. genital swabs collected from a human subject (*Homo sapiens*). We find that cotton and viscose swab materials yield comparable results, and that baking these materials is an effective pre-cleaning treatment. Concerning column types, we find that a polyethylene glycol (PEG)-type GC column is more suitable for a wider range of compound polarities compared to a polysiloxane-type column. Next, we find that direct thermal desorption is comparable to other solventless extraction approaches. Finally, we find that sample queue time affects sample recovery. In sum, our methods promise to make scent sampling more practical and reproducible, enabling more effective scent research to further elucidate the field of animal communication.

## Finding the "needle in the haystack": a comparison of statistical tools for untargeted chemical analysis

Brigitte M. Weiß (1,2), Erik Kusch (3,4), Marlen Kücklich (1,2), & Anja Widdig (1,2,5)

(1) Institute of Biology, University of Leipzig, Germany

(2) Research Group "Primate Behavioural Ecology", Department of Human Behaviour, Ecology and Culture, Max-Planck-Institute for Evolutionary Anthropology, Leipzig, Germany

(3) Center for Biodiversity Dynamics in a Changing World (BIOCHANGE), Section for Ecoinformatics & Biodiversity, Department of Biology, Aarhus University, Denmark

(4) Spatial and Population Ecology, Institute of Biodiversity, Animal Health & Comparative Medicine, University of Glasgow, Scotland, UK

(5) German Center for Integrative Biodiversity Research (iDiv), Leipzig, Germany

Deciphering chemical cues and signals frequently resembles the search for a "needle in a haystack" of chemical compounds. Particularly in non-model vertebrate species, the complexity of chemical profiles combined with little prior knowledge about potential semiochemicals leads to various challenges, including statistical analysis. Statistical tools need to be able to pinpoint accurately which compounds out of hundreds of candidates are associated with certain biological traits. We therefore investigated eight commonly used statistical methods (Partial least squares, Random Forest, Procrustes and Similarity Percentage analysis, Analysis of Similarity, Mantel test, permutational MANOVA and GLMM) for how they perform under the challenges of untargeted chemical analysis. We created more than 2000 simulated data sets that systematically varied in sample size, the number and intensity of compounds in a chemical profile, the number and intensity of compounds associated with a trait, the presence of noise, and the presence of repeated measures. Four discrete and four continuous traits were included, half of which contained simulated associations with certain compounds while the other half did not. For each data set, we determined which compounds were found to be associated with these traits and compared the test results to the actually simulated associations. False positives were close to the nominal level for all tests, but false negatives varied. GLMMs with vectorized data matrix showed high sensitivity (i.e. few false negatives) even when few or relatively small compounds were associated with a trait. Along with Random Forest analysis, these GLMMs also performed best in pinpointing which of the compounds were associated with the respective trait. Finally, we applied all statistical tests to real mammalian chemical profiles. Overall, results suggest that combining two to three statistical tests (e.g. GLMM, Analysis of Similarity, Random Forest Analysis) covers different analytical aspects best and allows for the most robust conclusions.

## Evaluation and contextualisation of volatile organic compounds from breath samples of type 1 diabetes during hypoglycaemia, hyperglycaemia and euglycaemia.

Zoe Parr-Cortes (1), NJ Rooney (1), M Wheatstone (3, 2), T Stock (3), C Pesterfield (2), C Guest (2) & CT Müller (3)

(1) Bristol Veterinary School, Bristol, United Kingdom,

(2) Medical Detection Dogs, Milton Keynes, United Kingdom,

(3) School of Biosciences, Cardiff University, United Kingdom

Tight control of blood glucose reduces the risk of severe health consequences that can accompany poor management of Type 1 Diabetes. The search for non-invasive monitoring systems has seen an increase in research into breath analysis to detect changes in volatile organic compounds (VOCs) associated with blood glucose fluctuations. Diabetes Alert Dogs have been shown to accurately alert owners with Type 1 Diabetes to changes in blood glucose, sometimes before blood glucose monitors are able to detect a change and are likely responding to odours in breath as well as possibly perspiration. Objectives: To compare VOCs of breath samples between individuals with and without diabetes and the effects of blood glucose concentration, collection material, length of storage and acute illness on samples from an individual with Type 1 Diabetes. Methods: We conducted a preliminary study using breath samples from an individual with Type 1 Diabetes collected during euglycaemia, hypoglycaemia and hyperglycaemia and from an individual without diabetes. Samples were analysed using thermal desorption gas-chromatography time-of-flight mass-spectrometry (TD-TOF-GC-MS). A combination of statistical methods including Permutational Multivariate Analysis of Variance, Canonical Analysis of Principal Coordinates, Random Forests and Weighted Gene Co-expression Network Analysis were used to determine whether VOCs differed between groups. Results: Significant differences were seen in VOC profiles between participants with and without Type 1 Diabetes, and within the latter at different blood glucose levels. Variables such as collection material, acute illness and length of storage were also found to have a significant effect on the VPC profiles. Conclusions: Differences in VOC profiles associated with blood glucose levels could explain how Diabetes Alert Dogs detect and alert to changes in an individual following odour training. Differences associated with storage, collection material and acute illness have practical implications for dog training and dog performance.

## Life scientists in chemical ecology can benefit from psychology's responses to its own 'reproducibility crisis'

Tristram Wyatt

Department of Zoology, University of Oxford, UK

Reproducibility failures have affected all parts of the life sciences relevant to CSiV, from animal behaviour to molecular biology. Psychologists have responded constructively and creatively to their own field's very public 'reproducibility crisis'. The solutions include new ways of doing experiments, such as Registered Reports and aspects of Open Science. To illustrate some of our own challenges in chemical ecology, I will use the story of the 'putative human pheromones' androstadienone and estratetraenol which, despite never having been shown to be pheromones, have been the subject of some 60 studies claiming 'significant' positive results. These are quite possibly false positives, part of the 'reproducibility crisis', sadly common in the rest of the life and biomedical sciences, which has many instances of whole fields based on false positives. Chemical ecological research would benefit from vigorously adopting the proposals made by psychologists to enable better, more reliable science, with an emphasis on enhancing reproducibility. A key change is the adoption of study pre-registration and/or Registered Reports, which will also reduce publication bias. A growing number of journals covering chemical ecology offer Registered Reports, including *BMC Biology*, *Nature Communications*, *Scientific Reports*, and *Royal Society Open Science*.

## Keynote

### Enzymatic systems involved in peri-receptor events of olfaction in Mammalia: evolutionary perspectives

Fabrice Neiers

Centre for Taste, Smell and Feeding Behavior Science, CNRS, Inrae, Université de Bourgogne Franche-Comté, Dijon, France

The olfactory epithelium is continuously exposed to exogenous chemicals, including compounds bearing odor activity. Organisms have developed multiple mechanisms for the protection of this epithelium, among which antimicrobial proteins, enzymes fighting the oxidative stress and xenobiotic metabolizing enzymes. Our approach consists in characterizing by immunohistochemistry and mass spectroscopy analysis the proteome of the nasal mucus in different mammals (currently including Harbour porpoise (*Phocoena phocoena*), *Homo sapiens*, *Mus musculus*, *Oryctolagus cuniculus*, *Rattus norvegicus*). These analyses reveal some core enzyme families in the different species investigated, including glutathione transferases (GSTs). GSTs represent a large family of enzymes comprising numerous members that appear ubiquitous in terrestrial organisms. GSTs operate in catalyzing the conjugation of glutathione (present in the nasal mucus) with various compounds including odorants, leading to their elimination and thus playing a role in the termination of the olfactory stimulus. Our results support the function of GSTs in the peripheral olfactory process, in terms of modulation of odorant availability for the olfactory receptors. We will explore the expression and function of the GST enzymes in different species of mammals and their redundant functions in the olfactory system. These results on mammalian GSTs will be discussed in an evolutionary perspective in comparison with insect GSTs. Although insects have evolved a distinct olfactory system from mammals, their GSTs have a similar role in olfaction.

# Thematic Session IV

## Chemical signals in a multisensory environment

### Chairs

Isabelle Charrier (1) & Arnaud Leleu (2)

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In cognitive and behavioral sciences, sensory modalities are generally investigated in isolation. However, living beings evolve in a rich environment replete with inputs coming from all modalities at the same time, and individuals must integrate myriad information across the senses to efficiently perceive this environment and adapt their behavior accordingly. How do the senses communicate with each other for a unified perception of the environment? What is the contribution of the chemical modalities to this merging of sensory information? The speakers of the present symposium will provide answers to these questions by drawing upon research conducted in four different species, birds, pinnipeds, and two primates. In particular, they will demonstrate the role played by the chemical signals in multisensory contexts and illustrate commonalities and dissimilarities across the senses for each species. This symposium will thus shed new light on the specificity of the chemical senses in the multisensory processing of information and underline how living beings utilize (chemical) sensory information to make sense of their surroundings.

### Speakers

Speaker #1 Simon Potier

Vision Group, Lund University, Sweden

***Insight into the sensory ecology of raptors: Vision and olfaction***

Speaker #2 Diane Rekow

Laboratoire Éthologie Développementale et Psychologie Cognitive, Centre des Sciences du Goût et de l'Alimentation, CNRS - Université Bourgogne Franche-Comté – Inra – AgrosSup Dijon, Dijon, France

***How odors assist the developing visual system in humans***

Speaker #3 Christine Drea

Department of Evolutionary Anthropology, University Program in Ecology, and Duke Lemur Center, Duke University, Durham, North Carolina, USA

***The 'language' of scent within multimodal lemur signals***

Speaker #4 Ben Pitcher

Marine Predator Research Group, Department of Biological Sciences, Macquarie University, Sydney, Australia – Taronga Institute of Science and Learning, Taronga Conservation Society, Sydney, Australia.

***Multimodal recognition of the pup by Australian sea lion mothers***

## Insight into the sensory ecology of raptors: Vision and olfaction

Simon Potier (1), Olivier Duriez (2, 3), Aurélie Célérier (2, 3), & Francesco Bonadonna (2, 3)

(1) Vision Group, Lund University, Lund, Sweden

(2) CEFE UMR 5175, CNRS-Université de Montpellier - Université Paul-Valéry Montpellier, France

(3) EPHE - 1919 Route de Mende, 34293 Montpellier, France.

Raptors are believed to rely mostly upon vision for their daily tasks. Especially, the fact that some species can fly at high altitude, such as vultures, have led scientists to consider that raptors have extraordinarily sharp eyesight. However, animals experience a variety of different ecological conditions throughout their lives, which may favour the use of different sensory abilities, even in those organisms that are believed to rely predominantly on one sense such as raptors. I will present here a number of evidences that some raptors can use the sense of smell to forage. Then I will present the relative importance of vision and olfaction in a foraging context for two species with different ecology, the obligate scavenger Turkey vulture *Cathartes aura* and the opportunist Southern caracara *Caracara plancus*. Olfaction may be more important in raptors than previously thought. However, information about this sensory modality remains extremely scarce in this group, and the multimodal association between olfaction and other sensory modalities need to be studied in detail.



## How odors assist the developing visual system in humans

Diane Rekow (1), Arnaud Leleu (1), Anna Kiseleva (1), Karine Durand (1), Benoist Schaal (1), Bruno Rossion (2, 3) & Jean-Yves Baudouin (4)

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(4) Laboratoire "Développement, Individu, Processus, Handicap, Éducation" (DIPHE), Université de Lyon (Lumière Lyon 2), 5, avenue Pierre-Mendès-France, 69676 Bron, France

Sensory perception allows us to rapidly interpret our surroundings with apparent simplicity and automaticity. To achieve that, our brain notably relies on categorization, i.e., stimuli dissociated into categories (discrimination) and grouped together within categories (generalization). For the developing human brain, the challenge is plural: we evolve in a multisensory environment and our senses convey a continuous flow of varied and variable information. The questions we will tackle in this presentation are: How does multisensory perceptual categorization develop across the senses? In that respect, are all senses equal? To provide answers, we will focus on the case of multisensory perception of conspecifics, and reveal olfactory-visual interactions in young infants, using some of the most salient inputs for a social species such as humans: body odors and faces. In a set of studies using non-invasive scalp electroencephalography (EEG), 4-to-12-month-old infants were tested while they were presented with streams of images and visual categorization is measured using a frequency-tagging approach. During 30-sec-long visual sequences, 6 images were presented per second (i.e., various objects lasting 167 ms each) and a target category (e.g., a face) was introduced every 6th stimulus to measure a category-selective neural response. In the meantime, infants were alternatively exposed to a contextual odor: their mother's body odor or a baseline odor. This multisensory interaction was tested for face and nonface stimuli and compared across odor contexts over the 1st year of life. This paradigm allows to measure the effect of olfactory-visual congruency on visual categorization and revealed positive effects of congruency for the olfactory-visual interaction and a trade-off between the contribution of the senses. Findings will be discussed with regards to the role of odors in the construction of visual categorization, focusing on the strong saliency conveyed by conspecifics for the young infant brain.

## The 'language' of scent within multimodal lemur signals

Christine M. Drea (1,2) & Lydia K. Greene (2,3)

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(2) Department of Biology, Duke University, Durham NC, 27708, USA.

(3) Duke Lemur Center, Durham NC, 27705, USA.

Strepsirrhine primates often mix scents from different sources to deposit composite olfactory signals, while also incorporating visual and auditory elements into their marking behavior. Lemurs are thus prime candidates for chemical and behavioral studies of the structure and function of composite unimodal and multimodal signals. Because scent types are chemically distinct, mixing increases information content, but can also differentiate messages, prolong signal longevity, or even 'weaponize' scent by creating a poison. Multimodal elements differentially draw audience attention: flamboyantly and audibly deposited fresh signals immediately attract nearby recipients, whereas physical scarring of marked substrates create identifiable posts of decaying signals for future outgroup recipients. To further our functional understanding, we examined the composite/multimodal marking bouts of captive, male Coquerel's sifakas (*Propithecus coquereli*), that combine genital and sternal glandular secretions with urine and tree gouging. While alerting conspecifics to the presence of olfactory information, the latter likely also functions to increase scent retention in freshly exposed substrate. We recorded all behavioral elements in sequence, specifically noting foundational and terminal elements, in > 900 bouts, and analyzed the findings relative to male social rank and the prior olfactory presence of intragroup females. Dominant males deposited more composite bouts in the environment, with more sternal components (particularly as foundational elements), than did subordinates. Members of both ranks produced more complex composite signals when overmarking female scent, with dominant males incorporating more sternal and urogenital elements, consistently starting and ending bouts with sternal and urogenital marks, respectively. We suggest that male sifakas may differentially construct their composite signals to absorb/mask female scent and/or encode particular messages for different recipients, such that the richness of bout elements and deposition order may function as a flexible olfactory syntax.

Supported by the National Science Foundation (IOS 0719003) and Duke University Undergraduate Research Support

## Multimodal recognition of the pup by Australian sea lion mothers

Benjamin J. Pitcher (1,2), Kaja Wierucka (1,3), Robert Harcourt (1) & Isabelle Charrier (4)

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(2) Taronga Conservation Society Australia, Mosman, NSW, Australia

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(4) Université Paris-Saclay, Université Paris-Sud, CNRS UMR 9197, Institut des Neurosciences Paris-Saclay, Orsay, France

Amongst otariids, mothers regularly alternate foraging at sea and nursing bouts in the colony. Mothers and pups must reunite following each foraging trip, and are likely to use a combination of acoustic, visual and olfactory cues to locate and recognise individuals. In Australian sea lions (*Neophoca cinerea*) maternal care lasts for at least 17 months, and during this time mother and pups are frequently separated. We have explored the multimodal recognition system used to coordinate reunions. Using chemical and acoustic analysis coupled with behavioural experiments in the olfactory, auditory and visual modalities, we have identified the underlying mechanistic basis of recognition. Gas chromatography-mass spectrometry (GC-MS), was used to examine the chemical profiles of two different colonies and assess similarity of chemical fingerprints in mother-pup pairs. This analysis allowed us to examine whether a chemical basis for phenotype matching exists in this species. Our results showed no GC-detectible mother-offspring similarity in the overall chemical fingerprints, suggesting that direct familiarisation is a more likely mechanism used by Australian sea lion mothers for recognition of their young. Our analysis also demonstrates that colony differences are encoded within chemical fingerprints and appear to be highly influenced by environmental compounds. Behavioural experiments showed that both olfactory and acoustic signals convey reliable information to facilitate individual recognition and that visual cues facilitate at least class recognition. When presented in congruent and incongruent combinations we found that despite each sensory cue accurately conveying identity information when presented in isolation, in a multimodal setting their importance, function or role may change and seems to be driven by the costs and benefits of obtaining information resulting from the constraints imposed by the active space of cues.

## OPEN SESSION 2

# Olfactory processes: From ecology to cognition, through sex and imprinting

Open Session 2-1

## Captivity and Communication: Comparative Analysis of Scents from Wild and Captive Tamarins

Alice C. Poirier (1,2), Jacob C. Dunn (2,3,4), Mrinalini Watsa (5,6), Gideon A. Erkenwick (6,7,8), Amanda D. Melin (1,9), John S. Waterhouse (2) & Andrew C. Smith (2)

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(7) Department of Biology, University of Missouri St. Louis, Saint Louis, Missouri, USA

(8) Department of Molecular Microbiology, Washington University School of Medicine, Saint Louis, MI, USA;

(9) Department of Medical Genetics & Alberta Children's Hospital Research Institute, Cumming School of Medicine, University of Calgary, Calgary, Canada

Mammals use semiochemicals during territorial and resource marking, as well as to exchange signals during social interactions. Odorants may be direct by-products of essential biochemical pathways, derived from the diet and the environment, and/or produced by commensal bacteria. Accordingly, animals in captivity, which are subjected to artificial diets and environments, may produce a different range of semiochemicals than found in their wild counterparts; unfortunately, few studies have directly compared chemosignalling in wild and captive conspecifics. We begin to address this gap by investigating the effect of captivity on chemosignalling in the bearded emperor tamarin, *Saguinus imperator subgriseus*. Scent samples collected from eight wild tamarins and analyzed by headspace solid-phase microextraction-gas chromatography-mass spectrometry contained over twice the number of identified compounds as those collected from five captive tamarins. Wild and captive scent samples also showed a marked overall difference in their chemical composition. Our results, although based on small sample sizes, suggest that captivity alters primate chemosignalling, with potential implications for captive husbandry practices, including conservation breeding programs of rare species.

## Mental gland chemical composition and sexual variation in the Spanish terrapin (*Mauremys leprosa*) and preliminary comparison with other chelonian species

Alejandro Ibáñez (1,2), Albert Martínez-Silvestre (3), Dagmara Podkowa (1), Aneta Woźniakiewicz (4), Michał Woźniakiewicz (4) & Maciej Pabijan (1)

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Although chelonians (turtles, terrapins and tortoises) have a good ability to discriminate chemicals in the environment, studies on intraspecific chemical signaling in this vertebrate group are scarce. Besides a well-developed chemosensory system, chelonians possess integumentary glands – i.e. mental glands – that secrete chemosignals. A comprehensive study published by the authors last year examined sexual variation in chemical composition and structure in mental glands of the Spanish terrapin *Mauremys leprosa*. Chemical characterization of mental gland secretions in male and female *M. leprosa* was carried out using gas chromatography-mass spectrometry (GC-MS). Here, we discuss these recent findings in *M. leprosa* and add new data on other species of chelonians. Steroids (especially cholesterol) constitute the bulk of mental gland secretions. Carboxylic acids, alkanes, alcohols and carbohydrates were also present. Histological examination in *Mauremys leprosa* revealed that mental glands are well-developed in males but very reduced - and likely functionless - in females. Many compounds were shared by both sexes, however some differences were apparent. Compared to females, males had larger amounts of two carboxylic acids and one steroid. Behavioral tests are needed to confirm the roles of these compounds in chemical signaling. Pilot data on mental gland secretions in four other chelonians showed that the main classes of compounds were relatively similar across species. In a preliminary analysis, we focused on the potential differences in specific steroids. Data on mental gland composition is currently being analyzed from a larger number of samples and species in order to provide a robust assessment of the variation at the interspecific level.

Funding: Financial support was obtained from the Polish National Science Center (grant no. UMO-2017/25/B/NZ8/01498 to Alejandro Ibáñez).

## Father-partner body odour similarity: Perceptual, microbial, and chemical dimensions

Jan Havlicek (1), Lucie Kuncová (1), Robert Hanus (3), Jakub Kreisinger (1), Pavlína Kyjaková (3), Radka Bušovská (3), Lucie Schmiedová (1), Jitka Třebická Fialová (1), Dagmar Schwambergová (1), Kristýna Šípková (1) & Zuzana Štěrbová (1,2)

(1) Faculty of Science, Charles University, Prague, Czech Republic

(2) Faculty of Arts, Charles University, Prague, Czech Republic

(3) Institute of Organic Chemistry and Biochemistry, Prague, Czech Republic

Women tend to choose partners resembling their father in various characteristics such as facial shape and ethnicity. This effect appears to be modulated by the quality of the relationship with the father during childhood. Olfactory imprinting was previously reported in several mammalian species; however, no similar phenomenon was explored in humans yet. To test parent-partner similarity in body odour, we collected axillary body odour samples from 67 women's partners and fathers. The perceived body odour similarity was evaluated by female panelists using a 4-choice match-to-sample test. To evaluate similarity in body odour microbiome, we performed 16S rDNA sequencing. We further assessed chemical profiles by using GC×GC-ToF-MS. Each woman also completed questionnaires to assess relationship quality with her father during childhood (s-EMBU). Results showed that the raters evaluated partners' body odour samples as the most similar to the fathers' samples with a frequency higher than chance (35.5 % vs. 25 %). We used Euclidean distances to assess the similarity between male partner and father in their chemical profile and microbiome. We found that similarity in chemical profiles was considerably higher in partner-father dyads as compared to randomly generated dyads. Similar pattern was observed in the composition of microbiota. Contrary to our expectation, the quality of the relationship with the father had no influence on the perceived father-partner similarity. These results provide the first systematic evidence for olfactory imprinting-like effect in humans.

## Short, long and very long-term olfactory memory in mice

Richard Brown, Oliver K. Schnare & Kyle Roddick

Department of Psychology and Neuroscience, Dalhousie University, Halifax, Nova Scotia, Canada

Schellinck et al. (2001, *Chemical Senses*, 26, 663-672), developed a simple and reliable test of olfactory learning and memory in mice using a Pavlovian conditioned odour preference task. Mice learned to discriminate between two odours after 1-4 daily 10-min trials with the CS+ odour (sugar reward) and CS- odour (no sugar) over four days. In the odour preference memory tests, mice remembered the CS+ for up to 60 days after training. Brown & Wong (2007, *Learning & Memory*, 14, 134-144) found that C57 and DBA mice could remember the odour discrimination for up to 9 months after training. Mice up to 24 months of age could learn and remember the odour preferences and Alzheimer model mice showed no odour memory deficits. The results of these experiments will be reviewed, and new data presented on the effects of altering the parameters of the Pavlovian conditioning paradigm on memory. Mice (C57) given 8 CS+ and 8 CS- trials on one training day showed no odour preference during the acquisition stage, but showed a conditioned odour preference on 24 hour, 7-day and 30-day memory tests. Mice (B6/C3H) given 4 CS+ and 4 CS- trials showed a conditioned odour preference on 24 hour and 7-day memory tests (pilot study). The results suggest that different one-day training paradigms result in different memory strength. Such a one-day appetitive training test could be valuable for studying the neurobiological bases of short, long, and very-long term memory.

## Keynote

### Traumatic mating in amphibians: male frogs (*Plectrohyla*, *Hylidae*) use their teeth to inject females with SPF-pheromones

Lisa M. Schulte\* (1) & Franky Bossuyt (2)

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(2) Amphibian Evolution Lab, Biology Department, Vrije Universiteit Brussel (VUB), Pleinlaan 2, 1050 Brussels, Belgium

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#### Abstract

Courtship and sex pheromones are important components of successful mating in many animal species. In amphibians most known pheromones are proteins which are expressed and secreted from species-specific male breeding glands during the reproductive season. While a lot of studies have focused on chemical communication in salamanders, in anurans (frogs and toads) chemical communication during courtship has received much less attention so far. Trying to close this gap, we study courtship pheromones and the associated behaviours in different anuran species. One especially strange breeding behaviour can be found in some hylid frogs: the transmission of pheromones via traumatic mating. Traumatic mating is one of the most efficient ways to avoid signal loss to the environment during pheromone transfer. The chemical substances are applied directly into wounds inflicted with specialized devices to the mating partner. It has evolved multiple times in the animal kingdom, but in vertebrates traumatic mating has only been documented from salamanders so far. However, in several species of the anuran genus *Plectrohyla* (*Hylidae*), the males have elongated teeth and develop swollen upper lips during the breeding season. Behavioural observations revealed that males press their upper jaw onto the females' dorsum during amplexus, leaving small skin scratches with their teeth. Histological examinations identified glands in the males' lips, resembling known amphibian pheromone glands. Whole-transcriptome-sequencing of these glands showed high expression of sodefrin precursor-like factor proteins (SPF), which are known to have a pheromone function in other amphibian species, where they are typically transmitted over the nares. Our results suggest that SPF pheromones are delivered via traumatic mating in these frogs, i.e. are applied from the male lip glands via skin scratches. Our research not only supports a role for pheromone use during chemical communication in anuran courtship, it also shows different pathways of pheromone delivery in these animals.



# Friday 5 Nov

9.00-9.10: Welcome – novelties of the day

9.10-10.10: **PLENARY** Masao Miyazaki & Reiko Uenoyama Chair: Jane L Hurst  
The characteristic response of domestic cats to plant iridoids allows them to gain chemical defense against mosquitoes.

10:10 – 10:20 Break

10:20-11:49 **THEMATIC SESSION V** Chair: Michał Dzięcioł  
*Chemical signal of health disorders - the use of trained animals as biological detectors*

10:20-10:25 Introduction: M Dzięcioł

10:26-10:46 Can mice detect odour of neoplasm before clinical symptoms? A Kokocińska-Kusiak, A Matczuk & T Jezierski

10:47-11:07 Canine detection of cancer in humans - expectations and reality. T Jezierski & M Dzięcioł

11:08-11:28 PTSD alert dogs: Could dogs alert to the early onset of panic attacks and flashbacks in humans? S Gadbois & L Kiiroja

11:29-11:49 Trained dogs and chemical analysis demonstrate the existence of an epileptic seizure odour in humans. A Catala, H Cousillas, C Levasseur-Garcia, M Pagès, JL Schaff, U Till, L Vitola Pasetto, M Hausberger, J Cattet, F Violleau, M Grandgeorge

11:50 – 12:00 Break

12:00-13:16 OPEN SESSION 3

Chair: Jan Havlicek

*Intra- and interspecific communication, comparative aspects and relations with management*

12:01-12:19 Deciphering the potential of urine in the chemical communication of little Indian field mice, *Mus booduga*. D Sankarganesh, P Ponmanickam, S Muniasamy, T Rajagopae, R Lakshminarayanan Rengarajan, J Angayarkanni, S Achiraman, G Archunan

12:20-12:38 Who is "stronger": a cat or a mouse? Comparison of the effect of cat and mouse urine on maturation of Campbell's hamster (*Phodopus campbelli*) males. A Khrushchova, N Yu, AN Vasilieva

12:39-12:57 Specific antipredatory response to the smell of different species of the snake skin: Is it better to fight or flight for leopard gecko (*Eublepharis macularius*)? E Landová, P Frýdlová, A Chomik, P Hnidová, & D Frynta

12:58-13:16 Chemical Communication and Elephant Conservation: Chili Pepper Fences and Other Chemo-Approaches. B Schulte, R Lynn Von Hagen, M Githiru, U N. Mutwiwa, S Kasaine, B Amakobe & S Corde.

13:16 – 14:15 Poster session (daily suggested selection)

13 - Preferential processing of chemosensory satiety cues. AS Schäfer, BM Pause, M Hoenen, KT Lübke & U Stockhorst

14 - Association between self-reported and third party reported attractiveness in body odour. L Kunkova, J Třebická Fialová, D Schwambergová, Z Štěrbová, V Třebický, Ž Slámová, V Kunc, J Havlíček

15 - Maternal body odor helps the development of rapid face categorization in the human infant brain. A Kiseleva, B Schaal, D Rekow & A Leleu

16 - Perianal secretion marking behaviour of the European mink in the face of visual and olfactory signals by conspecifics and predators. L Ortiz-Jiménez, I Barja

17 - Analytical and behavioural characterization of body odour constituents in the songbird *Taeniopygia guttata*. T Alves Soares, BA Caspers, HM Loos

18 - A cases of atypical sexual attractiveness in a spayed male of domestic dog - a case study. M Woszczyło, A Szumny, J Łyczko T Jezierski, P Krzemińska, I Szczerbał, M Świtoński, W Niżański, M Dzieciot

**14:15-15:31 OPEN SESSION 4**

Chair: Matthieu Keller

***Chemoreception and reproductive processes***

14:16-14:34 Courtship pheromones in sexually dimorphic finger glands of the Neotropical poison frogs (*Dendrobatoidea*). D Abondano-Almeida, E Twomey, F Vargas-Salinas & LM Schulte

14:35-14:53 Molecular and cellular changes in vomeronasal organ alterations in animals. P Asproni, C Bienboire-Frosini, V Mechin, A Cozzi, C Chabaud, E Teruel & P Pageat

14:54-15:12 Effects of male axillary extracts on salivary LH, cortisol and mood in women. T Laktionova, I Kvasha & V Voznessenskaya

15:13-15:31 Integrative chemical ecology: emission and reception of chemosignals in mammals. P Nagnan-Le Meillour, C Le Danvic & M Keller

**15:35 -16:30 DUAL KEYNOTE**

Flora Gouzerh & Jane L Hurst

Chair: Guila Ganem

**Morbidity-related odors and social behaviour**

15:35-15:40 Introduction. G Ganem

15:41-16:01 Detection and preference of mice for smells of cancerous congeners. F Gouzerh, B Buatois , L Dormont, F Thomas, G Ganem.

16:02-16:22 Do changes in urinary protein signals allow female house mice to avoid infection with lymphocytic choriomeningitis virus (LCMV)? J L Hurst & R Beynon

16:23-16:30 Discussion

**16:30- 17:00 PLANNING THE NEXT CSIV MEETING**

**17:00 CLOSING OF THE 15TH CSIV MEETING**

# Day 3 Abstracts

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## Plenary

The characteristic response of domestic cats to plant iridoids allows them to gain chemical defense against mosquitoes

Masao Miyazaki & Reiko Uenoyama

Department of Biological Chemistry and Food Sciences, Faculty of Agriculture, Iwate University, Japan.

Domestic cats (*Felis silvestris catus*) and other felids show a characteristic response to certain plants such as catnip (*Nepeta cataria*) and silver vine (*Actinidia polygama*), which comprises licking and chewing the plants, face and head rubbing to the plants, and rolling on the plants. This response is induced by plant iridoids, nepetalactone (catnip) and dihydronepetalactone, iridomyrmecin, and these isomers (silver vine), but its biological significance and underlying mechanism remained undetermined. Here, we introduce our studies uncovering the neurophysiological mechanism and behavioral significance of this enigmatic response which was first observed over 300 years ago. We first isolated nepetalactol as a potent bioactive compound for cats from silver vine leaf extract, which had been missed in past studies. Nepetalactol also induces the characteristic response in jaguar and leopard. Plasma  $\beta$ -endorphin, an endogenous opiate, is markedly increased immediately after the response to only nepetalactol in cats. A treatment of naloxone, an antagonist of  $\mu$ -opioid receptors, inhibits the expression of the response. These indicate that the  $\mu$ -opioid system regulating euphoric and rewarding effects in humans is involved in the expression of the feline response. Furthermore, behavioral analyses demonstrate that rubbing and rolling on the leaves transfers nepetalactol to their head and body furs. Nepetalactol is repellent to insects such as mosquitoes as well as nepetalactone reported previously. As a consequence, iridoids transferred to feline's fur protect cats from mosquito bites. Our recent studies also find that cats have evolved sensitivity to plant-specific iridoid production. Feline licking and chewing damages catnip and silver vine leaves which increase emission of iridoids from the leaves. Damage to silver vine increases the complexity of leaves' iridoid profiles. Cats exhibit maximum responsiveness to damaged leaves to the amount and compositions of iridoids produced by plant iridoids, but these substantially differ between plant species; for catnip, cats respond to a large amount of nepetalactone and for silver vine, a much smaller amount of mixed iridoids. Our studies provide an important example of how animals utilize plant's metabolites for their pest defense through coordinated patterns of behavior *via* the  $\mu$ -opioid system.

This study was made possible thanks to the close collaboration of Profs. Jane Hurst and Rob Beynon.

## Thematic Session V

### Chemical signal of health disorders- the use of trained animals as biological detectors.

Chair: Michał Dzięcioł

Department of Reproduction, Faculty of Veterinary Medicine

Wroclaw University of Environmental and Life Sciences, Wroclaw, Poland

The subject of the proposed symposium is connected with the capacity of animals to detect changes in volatome of the persons suffering from various diseases. This capacity of the trained animals has been confirmed by many studies. Proposed speakers however are the high class specialists in the field of animal olfaction and are able to present also critical points of view regarding the related subject. Detection of health disorders with the use of chemical signals emitted by the organism as a consequence of metabolism modification is a well known issue. In the current situation, discussion about its mechanisms, effectiveness, usefulness, and other aspects, is supposed to be very timely, however, even apart from that, the subject seems to be interesting, worth paying attention to and considering in the thematic scope of the symposium.

Speaker #1 Dr Agata Kokocińska- Kusiak

Warsaw University of Life Sciences, Institute of Animal Sciences, 02-786 Warszawa  
ul. Ciszewskiego 8, Poland

***Can mice detect odour of neoplasm before clinical symptoms?***

Speaker #2 Prof. dr hab. Tadeusz Jezierski

Institute of Genetics and Animal Biotechnology of Polish Academy of Sciences,  
Department of Animal Behaviour and Welfare, Jastrzębiec, 05-552 Magdalenka,  
Poland

***Canine detection of cancer in humans - expectations and reality***

Speaker #3 Laura Kiiroja & Simon Gadbois

Canid and Reptile Behaviour and Olfaction Lab, Department of Psychology and  
Neuroscience, Dalhousie University, Canada

***PTSD alert dogs: Could dogs alert to the early onset of panic attacks and  
flashbacks in humans?***

Speaker #4 Amélie Catala

Association Handi'Chiens, Paris, France & Laboratoire EthoS, Université de  
Rennes, Normandie Univ, CNRS, Rennes, France

***Trained dogs and chemical analysis demonstrate the existence of an epileptic  
seizure odour in humans.***

## Mice training to discriminate urine odor of conspecifics with neoplasm before clinical symptoms

Agata Kokocińska-Kusiak, Anna Matczuk & Tadeusz Jezierski

Institute of Genetics and Animal Breeding of Polish Academy of Sciences, Department of Animal Behaviour, Magdalenka, Poland

Recently the ability of animals to distinguish odours of humans suffering from some diseases such as cancer, tuberculosis, as well as alerting episodes of hypoglycemia in diabetes type I or seizure was increasingly reported. The most widely studied species are dogs that were used in detection research like: lung cancer (Amundsen et al. 2014), breast cancer (McCulloch M., Jezierski T., et al. 2006), melanoma, (Pickel, Manucy, et al. 2004), colorectal cancer (Sonoda, Kohnoe, et al. 2011), bladder cancer (Willis, Church, et al. 2004), hypoglycemia (Hardin, Anderson, Cattet, 2015), seizure (Strong, Brown, Walker, 1999). It is also known that other species are able to distinguish odour of healthy and unhealthy individuals like rats in detection of tuberculosis (Edwards, Ellis, Watkins et al. 2017; Ellis, Mulder, Valverde et al. 2017) or mice, which can distinguish by odour parasite infections (Kavaliers et al. 2003; Ehman and Scott 2002), influenza (Penn et al. 1996), mouse mammary tumor virus (Yamazaki et al. 2002) and inflammatory processes (Arakawa et al. 2010). Pathological processes involve production of either new volatile organic compounds (VOCs), that were not present in healthy individuals, or changes in the proportion of particular VOCs. Trained mice have been shown capable to distinguish urinary odours from conspecifics with and without experimentally induced lung cancer tumors (Matsumura et al. 2010), however, until now we did not know the moment of the smell appearing. This study sought to determine whether mice can differentiate conspecifics with melanoma in early stages (no clinical signs are detectable) from healthy animals. Forty male mice of the B57BL6 strain were urine donors before and after orthotopic inoculation with melanoma cells. The group of 35 males and 31 females were either tested for spontaneous preference for urine odor from donors with and without melanoma in open-field and Y-maze tests. After checking mice spontaneous preferences, the Y-maze test was used in discrimination training. No significant spontaneous preference for urine samples from animals with melanoma or from healthy animals was observed. Mice were successfully trained to discriminate between urine samples from donors with melanoma (with or without clinical symptoms) versus urine from healthy controls ( $P < 0.001$ ). The study demonstrated that mice can discriminate the odor of melanoma in the urine of their conspecifics even before visible clinical melanoma symptoms appear.

## Canine detection of cancer in humans - expectations and reality

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The ability of dogs to detect tumors in humans was first reported in 1989 in medical journal *The Lancet*, describing a case of an untrained pet dog which due to its spontaneous peculiar behaviour contributed to detection of melanoma on the leg of its owner. A conference in the UK in 2003 triggered experimental training of dogs by researchers in several countries, for detection of different kinds of cancer like lung, breast, prostate, ovarian, colorectal and bladder cancers, on the basis of odour. A scent lineups or circles containing mostly breath or urine odour samples, in some studies also feces or blood or tissue samples, were used. A variety of dog breeds mostly Labrador Retrievers, Border Collies and mogrels were trained over 3-16 months in lineups of 4-10 odor samples, using operant conditioning. Most peer-reviewed studies were published in the period 2004-2014, however, the methodology was characterized by lacking standardization. The trained dogs discriminated odour samples collected from patients with cancers of different stages, from samples of healthy controls with a sensitivity ranging from no better than by chance to 100% (on average 75%) and specificity 20-99% (on average 81%). Numerous attempts using Gas Chromatography –Mass Spectrometry could not identify a single volatile organic compound (VOC) or a simple combination of VOCs as a cancer odour marker to which the dogs alert. Thus the canine detection of cancer remains a „black-box technology”. It is still not confirmed that dogs can sniff out cancers in early stages of the disease, before ultimate medical diagnosis. Although the training of „cancer detecting dogs” is offered by some dog trainers, there is still no confirmed proof that trained dogs could be used in oncology for cancer screening on a systematic basis and may contribute to decrease of cancer morbidity and mortality.

## PTSD alert dogs: Could dogs alert to the early onset of panic attacks and flashbacks in humans?

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Post-traumatic stress disorder (PTSD) is a mental health disorder with high prevalence among the military population, affecting an estimated 23% of post-9/11 US veterans. Among the general US population, lifetime PTSD prevalence is 7-8%. This has evoked the search for non-trauma-focused and less-stigmatizing complementary and alternative treatments that allow for an individualized approach. One such treatment is the use of PTSD service dogs, albeit limited by the relative lack of scientific validation of effectiveness. Our study aims to contribute to this inquiry by investigating whether dogs can detect putative stress-related volatile organic compounds (VOCs) in the breath of people with PTSD. In collaboration with another study, breath samples are collected from participants with PTSD during a calm state (control odour) and induced stress situation (target odour). The samples are collected in the form of medical grade face masks. Scent detection canines at Dalhousie University's Canid and Reptile Behaviour and Olfaction Lab are presented with the samples in 2AFC (two alternative forced choice) discrimination and go/no-go detection tasks to evaluate their ability to detect the target state (detection), and discriminate between the two states. The putative VOCs we expect the dogs to detect likely involve VOCs originating from the sympathetico-adreno-medullary axis (SAM; adrenaline, noradrenaline) and the hypothalamo-pituitary-adrenal axis (HPA; glucocorticoids). We discuss the use of Signal Detection Theory to assess the dogs' sensitivity and response biases, as well as a case-by-case (dog-by-dog and sample-by-sample) investigation of the scents based on human participation data. Evidence that dogs' olfactory capabilities enable detecting early human stress-response has a potential to improve PTSD service dog effectiveness and training procedures. Dogs' ability to detect secretions of the SAM axis informs training protocols to focus on alerting to the early onset of panic attacks and flashbacks, possibly even before the patients themselves are aware of it.



## Trained dogs and chemical analysis demonstrate the existence of an epileptic seizure odour in humans.

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The question is whether a “seizure-odour”, that would be transversal to individuals and types of seizures, exists. And if yes, whether it is present before a seizure. This would allow not only to detect the seizure when it is occurring, but also to predict it. Thus, the aim of this study was to test the existence of an odour associated with epileptic seizures using trained dogs and to go further by evaluating its dynamics using selected ion flow tube mass spectrometry (SIFT-MS). Five trained dogs were individually tested on a seven-choice task. At each trial (n=9), seven scent samples from one patient were presented in opaque cans: one from a seizure, two from a sports session, four taken on different days during calm activity. SIFT-MS was used to compare the odour fingerprint of the seizure samples with samples taken in inter-ictal state. Samples were classified using a Welch's modified t-test, and a classification tree analysis was then used to predict each patient's state. All dogs discriminated the seizure odour, for each person (n=5) involved in the study, with high sensitivity (67 to 100%) and specificity (95 to 100%). In addition, there was no learning during the test. SIFT-MS (n=14 patients) confirmed that epileptic seizures can be detected, and went further, showing that it was possible to predict these seizures by up to 4 h 37 min in advance with 92% and 75% of samples correctly classified in training and leave-one-out-cross-validation, respectively. This constitutes a first proof that, despite the variety of seizures and individual odours, seizures are associated with olfactory characteristics. These results open a large field of research both on fundamental and applied level, from a better understanding to mechanisms involved in a seizure to everyday life monitoring technologies such as service dogs or wearable electronic noses.

## Open session 3

# Intra- and interspecific communication, comparative aspects and relations with management

Open Session 3-1

Deciphering the potential of urine in the chemical communication of little Indian field mice, *Mus booduga*

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Rodents are one of the major pests of agricultural activities besides their adverse effect on human health by bearing harmful pathogens. The little Indian field mouse, *Mus booduga*, is widespread across India and other Asian countries. The measures to control this population are warranted owing to their notorious attributes. We hypothesize that understanding the semiochemical communication of *Mus booduga* would provide a better strategy to manage this population. This study analyzed the urinary volatile compounds of *Mus booduga* (i.e., adult male, adult female, and prepubertal male) adopting GC-MS analysis to identify putative pheromones. Besides, the urinary proteins were separated using SDS-PAGE, and proteins of interest were characterized using MALDI-TOF MS analysis to determine the presence of pheromone carrier proteins. GC-MS analysis revealed a total of 25 volatile compounds in the urine of mice, among which 11 were found to be present in all three groups of mice (male, female and prepubertal male). Furthermore, we have identified four unique compounds in female urine and six unique compounds in prepubertal urine. In contrast, no specific volatile compounds were found in male urine. SDS-PAGE analysis revealed three abundant proteins in male mouse urine (21 kDa, 23 kDa, and 66 kDa), which were identified as Selenium binding protein, Calcium-binding mitochondrial carrier protein ScaMC-1 like, and Serum albumin. With the above results, we suggest confirming that the pheromone property of the volatile compounds and further characterization of the urinary proteins shall shed light on the pheromone communication of *Mus booduga*. The results of the present study would prospectively be utilized to develop chemical formulations for eco-friendly rodent pest management.

## Who is "stronger": a cat or a mouse? Comparison of the effect of cat and mouse urine on maturation of Campbell's hamster (*Phodopus campbelli*) males

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It is documented that cat's urinary chemosignals have a strong influence on rodent behavior and physiology. The vast majority of studies was conducted on a house mouse (*Mus musculus domesticus*) traditionally used as a suitable model to study responses to cat odor. Indeed, the long term coevolution in a shared environment led to the development of mutual adaptations. One of such adaptations is, contrary to other studied species, the accelerative effect of cat urine on sexual maturation of juvenile male mice. Such an effect may at least partially explain an enormous reproductive success of this species. Ecological studies indicate that the house mouse may occupy different microhabitats and compete with other species, often becoming the dominant species in the ecological community. However, the mechanisms underlying high ecological plasticity and adaptiveness of the house mouse are still not fully understood. We suggested that mice urinary chemosignals may influence reproduction of other rodent species via urinary chemosignals. The aim of our study was to compare a possible effect of cat and house mouse urine odors on sexual maturation of Campbell's hamster males. Urine of two herbivorous rodent species, rat and golden hamster, were used as additional control odors. The results indicate that rat and golden hamster urine odors had no effect on maturation of Campbell's hamsters. At that, mice urinary chemosignals suppressed maturation of young Campbell's hamsters to the same extent as the cat's urine in simulated conditions of high predation risk. The data received indicate that mice may suppress reproduction of other rodents via odor cues. This, at least partially, explains the high competitiveness of the house mouse.

## Specific antipredatory response to the smell of different species of the snake skin: Is it better to fight or flight for leopard gecko (*Eublepharis macularius*)?

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Various snake species represent a significant source of predation for geckos. The danger of a snake is determined by several ecological factors, mainly its food specialization and foraging strategy, which are usually shared within monophyletic clades of snakes. Previous research revealed that the antipredatory reaction to the living snake is innate for leopard geckos and that there is no difference in the reaction to sympatric vs allopatric snake species. In this study, we carried out a confrontational test quantifying the antipredatory response of leopard geckos *Eublepharis macularius* to snake skin odour in several experiments: 1) we analyzed antipredatory reactions to the piece of shedded snake skin and compare it to the control stimuli (a piece of the plastic bag); 2) we compare reactions to the eight snake predators from the Boidae and Colubridae family; 3) to uncover the presence of semiochemicals in snake skin, we tested the reaction towards snake skin after odour removal by polar and non-polar solvent. We found that geckos react by antipredatory response only to the snake skin in comparison to the controls. The first reaction to the stimulus was tongue-flicking and its frequency positively correlated with the interest of the animal. Geckos exhibited reaction toward all snake stimuli but varied in the type and intensity of the reaction. Our study revealed proactive (high posture, attack) and reactive (avoidance, low posture) elements of defensive behaviour towards snake skins. Elevated reactions were observed towards ericine (*E. colubrinus*, *E. johnii*) species. Geckos exhibited a reaction towards skin after odour removal by polar solvent, but no reaction after non-polar solvent application. We did not find any effect of sex of the tested snake on the antipredatory reaction. Geckos reacted to all snake skins, nevertheless, they expressed different antipredatory responses to the smell of skin of subterranean snake species.

## Chemical Communication and Elephant Conservation: Chili Pepper Fences and Other Chemo-Approaches

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As human-elephant coexistence (HEC) is challenged across Africa and Asia, conservationists attempt to find new and practical methods to mitigate crop raiding by Asian and African elephants. Elephants are notable for their keen sense of olfaction and reliance upon chemical signals for social interactions. Chili peppers with high Scoville Heat Units (SHU) were investigated decades ago to deter elephants from entering fields, using peppers as a border crop, in fires and smoke bombs, and as components of deterrent fences. The construction of fences with cloths soaked in pepper extracts mixed with oil has become popular and widespread, yet their effectiveness is highly variable. In conjunction with a large-scale deterrent study in Kenya, we created a laboratory methodology to evaluate the “heat” of the chemical components retained in chili and oil mixtures utilized for these fences. Using liquid chromatography and mass spectrometry (LC-MS) technology we developed an experiment to evaluate the levels of SHU from capsaicin and dihydrocapsaicin (the main chemical components in chili peppers) that were retained from these mixtures. We evaluated three commonly used recipes and found that mixing with oil was an important factor for higher SHU retention, though boiling peppers in water first was unnecessary. We also found that allowing pepper particulates to remain in the mixtures provided no different SHU retention than filtering. Results from our field experiments showed that chili fences performed poorly compared to other deterrent methods, suggesting chili pepper fences alone are not a reliable deterrent to elephant crop raiding. Abiotic factors such as wind may have contributed to their lower than expected effectiveness. We are exploring variations and alternatives to these fences in our application of chemical ecology to a major conservation issue.

# Open session 4

## Chemoreception and reproductive processes

Open Session 4-1

### Courtship pheromones in sexually dimorphic finger glands of the Neotropical poison frogs (*Dendrobatoidea*)

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Sexual chemical communication in amphibians is mediated by pheromone secretion produced in sexually dimorphic glands. Among these pheromones, Sodefrin precursor-like factors (SPFs) are known to have a reproductive function by enhancing female-receptivity in salamanders. A recent study showed the expression of SPF in male tree frog breeding glands, suggesting chemical communication during mating behaviour in anurans as well. In Neotropical poison frogs (*Dendrobatoidea*) a very specific type of breeding gland can be found: adult males of several species develop macroglands in the IV finger referred to as swollen finger IV (SFIV). Likewise, some dendrobatoids display cephalic amplexus, a behaviour in which the male grasps the female's head with his arms, which results in the male's fingers being in direct contact to the female's mouth or nostrils. Although the SFIV seem to be related to the cephalic amplexus, it is currently not known whether they are specialized for pheromone production and if this group of frogs uses chemical communication for reproduction. Here, we investigate whether the presence of sexually dimorphic glands is related to protein pheromone production in the fingers of the stripe-throated rocket frog, *Leucostethus brachistriatus*, a species that develops SFIV and displays a cephalic amplexus. Using a transcriptomic sequencing approach, we studied differential gene expression with an emphasis on examining the expression of SPFs associated with the presence of glands in the fingers of adult males. We found significant differential expression of SPF in the swollen fingers compared to the fingers lacking the glands. These results support the hypothesis that dendrobatoid species with swollen fingers use pheromonal signalling during courtship and further the evidence of chemical communication in *Dendrobatoidea*. Our findings open a whole new set of research questions regarding the role of the pheromones during mating, as well as their evolution within this diverse clade of frogs.

## Molecular and cellular changes in vomeronasal organ alterations in animals

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The vomeronasal organ (VNO) plays a crucial role in animal behaviour since it is responsible for semiochemicals detection, thanks to the vomeronasal sensory epithelium (VNSE). We previously described that VNO can be affected by inflammatory changes, and that VNSE inflammation is associated with behavioural disorders in cats and pigs, as probable consequences of intraspecific chemical communication impairment. This study aimed to investigate molecular and cellular alterations that appear in the VNSE when the VNO is affected by spontaneous changes, such as inflammation or aging processes. To study VNO inflammation, we analysed by histology and immunohistochemistry (IHC) 76 VNOs from 38 6-month-old farm pigs. Histology revealed that the VNSE was healthy in 13/76 VNOs (17%), weakly inflamed in 31/76 (41%) and moderately inflamed in 32/76 (42%). Pigs bearing VNSE inflammation were more involved in fights with other pigs ( $P < 0.001$ ). IHC showed that VNSE thickness and the number of *Gai2*-positive neurons were significantly reduced during inflammation and according to its intensity ( $P < 0.05$ ), meaning that inflammation led to neuron loss impacting VNO functionality. To study VNO aging, we selected the murine model (C57/6JRj and RjOrl:SWISS). We analysed 82 VNOs from 41 mice belonging to three ages: 3, 10 and 24 months (adult, middle-aged and old mice, respectively). VNSE microphotographs were analysed by ImageJ and revealed that aging was associated with neuronal degeneration ( $P = 0.0005$ ) and glycogen accumulation ( $P < 0.0001$ ). Image analysis also showed that olfactory marker protein and *Gai2* expression decreased with age ( $P = 0.0436$  and  $P < 0.0001$ , respectively), while *Gao* expression was increased ( $P < 0.0001$ ) as well as apoptosis ( $P = 0.0425$ ). These data showed that aging effects strongly impact VNO sensory structure and capabilities, leading to potential deficits in chemical communication efficiency in old animals. These studies showed that the VNO is affected by spontaneous changes and pathologies that can influence animals' chemical communication and, thus, their behaviour.

## Effects of male axillary extracts on salivary LH, cortisol and mood in women

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Hormones changes across women's menstrual cycle (MC) may lead to changes in perception of chemical signals and hormonal responses to these cues. Extracts of male axillary secretions (EMAS) directly affect LH-pulsing and mood of women, advancing the onset of the next peak of LH after its application in the MC follicular phase (Preti et al.,2003). Current study aims to investigate EMAS effects on salivary LH, cortisol and mood in women depending on the MC phase. We tested healthy women of reproductive and premenopausal age (n=29) with an EMAS/control solution applied once for 2 hours either in the follicular or the luteal phase of the MC. LH monitoring continued for 8 hours, with saliva sampled every 10 minutes. Cortisol monitoring continued for 4 hours, with saliva sampled every 20 minutes. LH and cortisol concentrations were analyzed using EIA technique. The Positive and Negative Affect Schedule (PANAS) and VAS were used to assess mood. We found that the frequency of LH peaks is affected by the women MC phase (ANOVA,  $F(1, 17)=45.37$ ,  $p=0.0000$ ,  $n=19$ ). We observed a significant increase in the number of LH peaks ( $p=0.0447$ ,  $n=10$ ) and of their amplitude ( $p=0.0469$ ,  $n=10$ ) when EMAS was applied to women in the follicular phase of MC. The same application in the luteal phase of MC did not affect the number of LH peaks ( $p=0.8379$ ,  $n=9$ ), but lowered its amplitude ( $p=0.0382$ ,  $n=9$ ). Salivary cortisol in women of reproductive age did not depend on the phase of MC (ANOVA  $F(1, 16)=1.0720$ ,  $p=0.3016$ ,  $n=18$ ). However, comparing reproductive age and premenopausal women, we found that the change of salivary cortisol under EMAS exposure depended on the age category of test subjects ( $p=0.0018$ ,  $n=28$ ). PANAS did not reveal any changes in positive or negative affects under EMAS exposure, neither in follicular nor in luteal phase of MC. Our data indicate the significance of the phase of MC for EMAS effects on LH and cortisol secretion, but not on mood in women.

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## Integrative chemical ecology: emission and reception of chemosignals in mammals

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The Chemical Ecology community has the objective to decipher the chemical communication of living animals, in order to use identified chemosignals for agricultural pests' management or to improve breeding systems in a fair way (clean, green, and ethical). In addition, a more recent objective is to identify chemosignals involved in the reproduction of endangered species to elaborate strategies for their protection.

The typical approach in Chemical Ecology consists in defining modalities of the exchange of signals in a given behaviour, identifying the potential chemosignals, doing chemical synthesis and performing behavioural assays with synthetic counterparts. This experimental strategy worked well with insects or some vertebrates, but is inappropriate to mammalian chemical communication. Indeed, a lot of chemosignals are not emitted by specialised glands, but are more likely by-products of the body metabolism, found in urine, sweat, and hair. This is thus difficult to identify specific signals from an olfactory background. There is a need for an alternative solution, such as reverse chemical ecology which sets up screenings of olfactory proteins involved in the reception of these signals, olfactory receptors (OR) and/or odorant-binding proteins (OBP). These screenings imply identification of the proteins, their expression in heterologous systems before electrophysiological assays (OR) or fluorescence quenching assays (OBP), and demand a lot of money and time.

We hereby present the identification of molecules emitted by males during the male effect in sheep and goats. We have also followed the composition of females' nasal mucus called olfactory secretome, which is composed of OBP isoforms. In order to select the potential semiochemicals, we propose a third way of screening, based on the analysis of the ligands of female's OBP.

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# Dual Keynote

## Morbidity-related odors and social behaviour

### Speaker#1

#### Detection and preference of mice for smells of cancerous congeners

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In many animal species, chemical communication plays a major role in individuals' interactions. As established for a large number of pathologies, cancer has been shown to induce changes in body odors. In the framework of research that aimed at finding non-invasive methods to detect early stages of cancer development, the present study asks whether untrained mice could detect the presence of cancer in odor sources of ill congeners, as they already do for some parasites. If yes, are they able to detect cancer at an early developmental stage? Which odorant molecules may be involved in this discrimination? Does it influence female sexual preference? Wild derived mice were involved in habituation/generalization and two-way preference tests, during which they were presented to odor stimuli of healthy versus cancerous mice (pulmonary adenocarcinoma). The stimuli were obtained from transgenic mice carrying different alleles of the EGFR mutation, for which cancer has been induced/or not following antibiotic inoculation. Our results indicate that mice can discriminate ill congeners at an early stage of cancer development. We also found quantitative differences suggesting that pheromones such as brevicomine and thiazoline were less present in ill versus healthy mice. Finally, the health status of males did not seem to influence female attraction to their smells, unlike studies involving smells of mice inoculated with intestinal parasites which were avoided by females. It would therefore seem that changes in body odor linked to cancer could be detectable by a mouse nose at an early stage of development, however these changes did not seem to influence female choice. If occurrence of cancer among mice in natural conditions mostly concerns old non-reproductive animals, our results suggest that selection did not favor evolution of discrimination against males carrying cancer.

# Dual Keynote

## Morbidity-related odors and social behaviour

### Speaker #2

Do changes in urinary protein signals allow female house mice to avoid infection with lymphocytic choriomeningitis virus (LCMV)?

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Sexually selected scent signals are widely used in mate choice. These are thought to advertise, to choosy mates, the signaller's quality such as their health status and ability to resist parasites and diseases. From the receiver's perspective, this may have direct benefits such as helping them to avoid pathogen transmission from infected animals, and indirect benefits gained from the inheritance of superior genes by their offspring. Nonetheless, we have little understanding of the impact of natural pathogens on scent signals or how signals may evolve to provide reliable information on infection status. Lymphocytic choriomeningitis virus (LCMV) is an endemic virus in house mice that induces only mild symptoms in adults. However, infection during pregnancy can have high costs, causing a reduction in embryo survival and the development of persistent lifelong infection among most embryos that survive. Females are at risk particularly during mating as infected males produce copious quantities of virions in their urine, faeces, saliva and semen. We have investigated the impact of acute or persistent LCMV infection on adult male production of major urinary proteins (MUPs), which are used for competitive sexual signalling in house mice (Oldstone et al. 2021 *Viruses* 13:1180). MUP output is substantially reduced during acute and persistent viral infection, though infection differentially affects production of the male sex pheromone darcin which induces female attraction, and the central MUP signature that signals a male's individual identity. However, males that have cleared an acute infection subsequently exhibit enhanced MUP production relative to uninfected control males. We will discuss the likely impact of these infection-related changes in MUP signals on female mate selection and the implications of LCMV infection for male signalling strategies and reproductive success.

# Posters

P-01

## Impact of diets unbalanced in n-3 polyunsaturated fatty acids on the mouse olfactory function

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In mammals, the peripheral olfactory system (also called olfactory mucosa (OM)) is composed of the olfactory epithelium and the underlying lamina propria. The olfactory epithelium contains several cell types including olfactory sensory neurons (OSNs) that detect the odorant molecules present in the surrounding environment. These neurons play a crucial role in the shaping of the olfactory information and its conveying towards the brain. Numerous works on rodents have shown that dietary deficiency in n-3 polyunsaturated fatty acid (PUFA) alters the functional properties of neural tissues such as the brain and the retina. In contrast, little is known about the effects of dietary PUFAs on the physiology of olfactory tissues. Therefore, the objective of our study was to investigate the impact of diets unbalanced in n-3 PUFAs on the composition and the functionality of OM in young adult mice. Female mice were fed with diets either deficient (LOW diet) or supplemented in n-3 PUFAs (HIGH diet) during the perinatal period. Then, the weaned male offspring were fed with the same diets for 5 weeks. At 8 weeks of age, olfactory behavior tests were performed and young adult mice were sacrificed in order to collect OM. The OM fatty acid profile as well as the expression of genes involved in different cellular pathways were evaluated. The OM electrical potentials (electro-olfactograms, EOG) in response to two odorants were recorded to assess the impact of diets on OM functionality. Compared to control mice, the LOW and HIGH diets significantly modified the fatty acid composition and the expression of genes involved in olfactory signaling and in OSN maturation in offspring OM. The amplitude of EOGs was reduced in mice fed with the LOW diet. However, mice olfactory behavior was not affected. In conclusion, our study showed that olfactory capacities of mice fed diets unbalanced in n-3 PUFAs during the perinatal period were maintained despite significant molecular and functional changes at the periphery of the olfactory system.

## Detection of buck olfactory pheromones by goats: a calcium imaging approach

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In most mammals, socio-sexual and reproductive behaviours are modulated by chemical signals (pheromones) picked up by sensory neurons of the olfactory system. Goats are seasonal breeders characterized by an anoestrus period in spring. However, anoestrus females recover sexual activity after exposure to a sexually active male (a phenomenon called "male effect"). This effect partially depends on olfactory cues emitted by the male. Indeed, exposure to active male fur reactivates the HPG axis, ultimately leading to LH secretion (Sakamoto et al., 2013). Whether females can respond to male odours only during their anoestrus period or all over the years is still unknown. To answer this question, we have developed a Ca<sup>2+</sup> imaging approach in goat olfactory neurons to identify and characterize neuronal populations detecting male odors. First, we freshly dissociated the main olfactory epithelium (MOE) and the vomeronasal organ (VNO) of female goats with papain to obtain single olfactory sensory neurons. Next, using a synthetic Ca<sup>2+</sup> sensor (fura-2) and epifluorescence microscopy, we established a live-cell Ca<sup>2+</sup> imaging protocol to determine the activity pattern of the sensory neurons in response to olfactory stimuli. We examined neural responses to male fur extracts, male urine, and synthetic organic compounds previously identified in these sources. All stimuli were tested in both MOE and VNO neuron preparations independently. We combined Ca<sup>2+</sup> imaging with immunocytochemistry of specific molecular markers (Omp and Gai2) by superimposing the activity map revealed by Ca<sup>2+</sup> imaging and the immunolabeling to characterize pheromone-detecting neurons. We tested prepubescent, ovariectomized, seasonally anoestrus, and pseudopregnant females. It would allow us to understand whether pheromone responsiveness can be affected by physiological status in a domestic model with seasonal breeding.

## Evaluation of feline semiochemical interactions with Cat vomeronasal type-1 receptor members using the multiple-ligand simultaneous docking (MLSD) approach

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The semiochemicals are detected by the peripheral receptors in the vomeronasal organ (VNO) for enhancing socio-sexual communication of the domestic cat. The vomeronasal type-1 receptors (V1R) belong to the superfamily of G-protein coupled receptors (GPCR) with seven transmembrane proteins (TM). V1R is playing a crucial role in perceiving the semiochemical cues to mediate species-specific responses in cats. Previously, the cat V1R9 structure was modelled and the residual interactions with the feline semiochemicals (CAP, FFP and FIS) were predicted using virtual screening. In this study, we have used a novel computational strategy to discover the efficient combination of feline pheromones with FELCATV1R9 using Multiple Ligand Simultaneous Docking (MLSD). The MLSD method is used to activate the interplay of multiple molecular interactions with the receptor binding sites. Our preliminary results revealed that the favorable ligand complex of feline semiochemicals showed more binding-free energy, frequency of binding and conventional H-bond interactions. Additionally, these ligand complexes revealed the similar ligand binding poses, fragment superimpose and ligand repositioning with the catV1R receptor. Thus, this dynamic approach could be very interesting to predict the best-fit ligands with the future pharmacophore modeling analysis.

## Sex differences in mice exploratory behaviour to Fel D 1, a cat ABP-like protein

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Fel d 1 is a cat protein from the secretoglobin family abundantly released and found in their habitat. It is closely related to mouse androgen-binding proteins (ABPs) and both have a common ancestor (Durairaj et al., 2018). We hypothesized that mice could detect and avoid this protein closely related to their own ABPs in a similar way as they do for Fel d 4, which is closely related to major urinary proteins (MUPs)(Papes et al., 2010). We tested purified natural Fel d 1, 2,5-Dihydro-2,4,5-trimethylthiazoline (TMT, positive control) and a blank in three different mouse groups (n=14 each) to evaluate exploratory behaviour and stress responses. We used a three chambered device for behavioural testing, the treatment was placed randomly in one of both sides and video recorded during 10 minutes (Grau et al., 2019). The mice did not show clear avoidance or stress responses to Fel d 1. Our results showed a sex x treatment interaction for Fel d 1, with males spending more time in the areas treated with Fel d 1 than in the untreated areas, which was not observed in females. This sex x treatment interaction was also not observed for either the blank or TMT. In another sex x treatment interaction, all groups showed shorter average times per passage in the untreated area than in the TMT area except for the Fel d 1 females, where there was no difference. These results showed that Fel d 1 from domestic cats elicit different responses in male and female mice. These sex differences could be linked to the sexual role of ABP proteins and the ABP-like characteristic of Fel d 1.

## Sensory detection by *Gai2*<sup>+</sup> VSNs modulates experience-dependent social behaviors in female mice

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In mammals, the olfactory system modulates reproductive and socio-sexual behaviors mainly by detecting chemosignals (including pheromones) by dedicated neurons of the vomeronasal organ (VNO). At least two populations of VNO sensory neurons (VSNs) detect pheromones through two families of G-protein-coupled receptors, V1Rs and V2Rs. Whether animals are intrinsically sensitive to the smell of conspecifics or detecting olfactory cues modulates experience for displaying social responses is currently unclear. Using a conditional knockout mouse for *Gai2* gene in all olfactory marker protein (OMP)-expressing cells, we showed that *Gai2* is required for sensory transduction in V1R-expressing vomeronasal neurons (Trouillet et al., 2019). Males deficient for *Gai2* signaling in VSNs present an impaired aggressive response toward other males or neonates, suggesting that *Gai2*<sup>+</sup> VSNs participate in male and pup odor detection. In female mice, olfactory-driven responses may evolve after social experience. For example, virgin females recognize pup odors and retrieve isolated pups to the nest to some extent, but maternity significantly increases this type of parental behavior. Similarly, the display of sexual receptivity is considerably enhanced by experience. Here, we reemployed our conditional knockout mouse model to show that social behavior in female mice results from interactions between intrinsic mechanisms in the vomeronasal system and experience-dependent plasticity (Trouillet et al., 2021). In pup- and sexually-naïve females, *Gai2* deletion elicited a reduction in pup retrieval behaviour, but not in sexual receptivity. By contrast, experienced animals showed normal maternal behavior, but the experience-dependent increase in sexual receptivity was incomplete. Further, lower receptivity was accompanied by reduced neuronal activity in the aAOB and RP3V. In this latest region, kisspeptin neuron activation was unchanged. Altogether, our data suggest that the detection of pheromones by the VNO influences olfactory-mediated behavior in females after social experience, although with distinctive traits for different behaviors.



## Painting inguinal wax on artificial nipples enhances weight gain in artificially fed lambs

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Ewes (*Ovis aries*) convey several odor substrates that seem to optimize lambs' initial guidance to the udder and to initiate suckling engagement (1). In particular, they produce a scented wax from a bilateral peri-mammary inguinal gland. This inguinal wax (IW) flows downward to the teats. When presented to unsuckled lambs, IW elicits positive orientation, intensified sniffing and oral seizing attempts (2). This IW is attractive/appetitive to newly born lambs before any contact with the udder and suckling experience (2). Artificially-fed lambs do not benefit from the IW's guidance and appetitive boost in their first attempts to interact with an artificial nipple. Thus, the nipple location and acquisition of sucking skills may be complicated in such lambs, and their learning of the artificial feeding context uneasy. To evaluate whether IW odor may improve sucking initiation and consequential growth in the artificial feeding context, we performed three experiments on Romane lambs (aged 12 to 24 h, i.e. after a first sucking experience). 1/ A paired-choice test to assess lambs' orientation (n=50) toward a IW-painted nipple vs. a control nipple; 2/ A suckling learning test consisting in introducing a nipple into the lambs' mouth during 3 s, 14 lambs being exposed to an IW-painted nipple, while 14 were exposed to a control nipple. 3/ A 3-week monitoring of growth in 15 lambs reared with an artificial milk feeder with IW-painted nipples and in 15 lambs reared with unscented control nipples. Results: 1/ The lambs' orientation was not significantly different toward IW and the control stimulus. 2/ No facilitation effect of IW odor was evidenced on learning to suck artificial nipples. However, 3/ the exposure to IW odor in artificial rearing significantly enhanced the daily growth rate of lambs. In the present conditions, IW odor did not show any attractive/appetitive effects on lambs. This may be due to the fact that the lambs were tested after they were induced to suck colostrum from their mother. Their better growth in the artificial rearing context in presence of IW-scented nipples may be due to a positive emotional effect induced by the continuous occurrence of IW odor on the feeder. This experiment needs replication to fully verify the validity of the hypothesized emotional effects of IW odor in relation with improved welfare in artificially-reared lambs.

## The testosterone-dependent felinine production is upregulated by an inhibition of the cholesterol biosynthesis in mice

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The domestic cat excretes a sulfur-containing amino acid known as felinine at a high concentration in the urine. Felinine is a precursor of volatile 3-mercapto-3-methylbutanol (MMB), which is a chemical signal for species and sex recognitions in cats. Our previous studies found that felinine is produced via a shunt of the mevalonate pathway for cholesterol biosynthesis in mammals, strongly suggesting that the primary function of the felinine biosynthesis is the down-regulation of the cholesterol biosynthesis to prevent hypercholesterolemia and then co-opted to produce MMB in cats. However, little is known why most of an intermediate of the cholesterol biosynthesis is metabolized to felinine in the feline specific manner. This study investigated the mechanism of the felinine biosynthesis using mice that excrete a felinine metabolite, N-acetyl felinine, at a low concentration. Since the felinine excretion level is much higher in male cats than female cats, we first examined sex differences of N-acetyl felinine excretion and the effect of administration of testosterone on its excretion in mice. N-Acetyl felinine excretion of male mice was significantly higher than that of female mice. The excretion of female mice administered testosterone for 10 days was increased to the same levels of that of male mice. Next, we treated mice using an inhibitor of farnesyl diphosphate synthase (FDPS) which produces cholesterol intermediates from end-products of the mevalonate pathway. N-Acetyl felinine excretion was much higher in FDPS-treated mice than control mice whereas plasma cholesterol levels were not varied between FDPS-treated and control mice. These results suggest that the felinine biosynthesis is regulated by testosterone in cats and mice, and the increment of end-products of the mevalonate pathway is the trigger for the acceleration of the felinine biosynthesis in mammals.

## The lip-smacking response of male dogs toward vaginal secretion and urine of estrous female dogs

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Male dogs (*Canis lupus familiaris*) detect estrous female dogs by sensing volatile compounds emitted from their vaginal secretion and urine. Previous studies reported methyl paraben in vaginal secretion of estrous females as a sex pheromone which induced sexual behavior in males. However, a controversy exists as to its bioactivity and content in excreted estrous urine, a mixture of vaginal secretion and urine. To identify sex pheromones from estrous vaginal secretion and urine, this study aimed to find indicators of behavioral responses of male dogs toward estrous vaginal secretion and urine. We also analyzed the content of methyl paraben in headspace above the urine samples collected from estrous and diestrous females. Male subjects exhibited sniffing and licking the secretion, excreting nasal mucus occasionally, and smacking their lips, quickly opening and closing the mouth with a sound, for a few seconds. Males showed a similar response toward the urine by over-marking their urine onto the estrous urine. Vaginal secretion, but not urine, kept its bioactivity after the freeze-thaw process. In gas chromatography-mass spectrometry analysis of volatile compounds emitted from female urine samples, methyl paraben was detected in both estrous and diestrous urine samples and its content did not differ significantly between the samples. Synthetic methyl paraben induced no specific behavioral response in the tests using estrous vaginal secretion and urine. In conclusion, the specific behavioral response of male dogs toward estrous female secretion is smacking their lips for a few seconds, which is a useful indicator to purify bioactive compounds from the secretion.

## Licking and chewing silver vine leaves by domestic cats enhances their ability to gain iridoids for chemical defense against pest insects

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Domestic cats (*Felis silvestris catus*) exhibit a characteristic behavioral response to specific plants such as catnip (*Nepeta cataria*) and silver vine (*Actinidia polygama*), comprising licking and chewing the plants, face and head rubbing, and rolling over. This response is induced by iridoids such as nepetalactone and nepetalactol, which are major bioactive compounds in catnip and silver vine, respectively. Our recent study uncovered that rubbing against the plants transfers these iridoids onto the faces and heads of cats, where they act as mosquito repellents. However the reason for licking and chewing the plants is unknown. Do cats, which are obligate carnivores, eat the plants? This study aimed to elucidate the function of licking and chewing silver vine leaves by cats. Licking and chewing silver vine leaves resulted in only negligible loss of leaf mass. Further, cats responded to the headspace of the leaf lipid extract by licking even without naso-oral contact. This indicates that the primary function of licking and chewing is not for consumption of the plants. Since leaves damaged by feline licking and chewing emitted a stronger smell than fresh leaves, we next examined whether these behaviors promote the emission of iridoids. Nepetalactol and other iridoids such as dihydronepetalactone were significantly higher in the headspace over leaves that had been damaged by feline licking and chewing than over undamaged leaves. Moreover, dihydronepetalactone increased markedly in both the headspace and lipid extract of damaged leaves. The chemical composition of iridoids from damaged leaves evoked more prolonged responses in cats than those from fresh leaves. In conclusion, licking and chewing silver vine leaves both promotes the emission of iridoids from the leaves and also changes the chemical composition of the iridoids, which enhances rubbing against this plant to gain chemical defense by cats.

## Structural brain plasticity and olfactory function in a mouse model of congenital blindness

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Early sensory deprivation, such as early blindness, results in enhancement of the remaining non-visual sensory modalities associated with functional and anatomical brain plasticity. While auditory and tactile functions have been largely investigated, results regarding olfactory functions remained less explored and consistent. A few studies showed enlargement of olfactory structures in the brain correlated with enhanced olfactory functions in early blind adults. The present study aims to assess olfactory performance and to examine anatomical changes in the whole brain in young (PND 9) and juvenile (PND 24) mice using a mouse model of congenital blindness. Volumetric analyses were conducted on high resolution MRI images and these morphometric measures were compared between blind and sighted ZRDBA mice, obtained by breeding ZRDCT and DBA mice. In this mouse strain, half of the littermates homozygous for the Rx/Rax gene are born blind and the other heterozygous half are born normally sighted. One group (20 blind, 20 sighted) underwent two structural MRI scanning at PND 9 and PND 24, while the second group (20 blind, 20 sighted) went through behavioral tests, such as odor attractivity and preference tests towards social odors (maternal, paternal, male, female odors). Based on preliminary results, we hypothesized that: 1) Structural MRI analyses will show brain plasticity in PND 24 blind pups, including olfactory areas (olfactory bulb, piriform cortex), as well as orbital areas, the amygdala, and vision-related structures. The enlargement of these olfactory structures in the blind mouse brain would be associated with better olfactory function. 2) Brain plasticity will be observed only in vision-related structures with similar olfactory abilities in PND 9 blind pups. This research will bring a better understanding of sensory processing and underlying mechanisms of intramodal and crossmodal brain plasticity following sensory deprivation, as well as the consequent behavioural changes.

## Comparative anatomy of the olfactory system of Neotropical poison frogs and its coherence with chemical communication

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In many amphibian species chemical communication plays an important role, and pheromones can be crucial for the reproductive behavior of these animals. However, sexual chemical communication has not yet been studied in Neotropical poison frogs (*Dendrobatoidea*), even though in some species the males perform a special form of amplexus related to the presentation of specialized dimorphic breeding glands, that potentially secrete pheromones. Here we compare the olfactory system of several species of Neotropical poison frogs including animals with and without those specialized breeding glands and specified mating behaviors. The aim is to draw a connection between the importance of chemical communication and the organization of the olfactory system. Therefore, different species were histologically examined. The heads were cut transversely from snout to tail in 6 µm thick slices with a microtome and stained with Mayers hämatoxylin. The cuts were photographed and analyzed qualitatively. Preliminary results from the species *Allobates talamancae* and *Allobates kingsburyi* suggest differences in structures in the olfactory system especially in the cavum medium and isthmus. These results suggest a potential correlation between the glands and the olfactory system, which would strengthen the hypothesis of chemical communication in *Dendrobatoidea*. If there are obvious differences in the olfactory system of species which show potential pheromone producing glands or a sexual behavior that might be linked to chemical communication, in contrast to species that show neither of those attributes, it could be an indication that pheromones play a crucial role in the mating of Neotropical poison frogs, and therefore need further investigation and behavioral testing.

## Signal or cue – search for clues about the release mechanism of damage-released alarm substances

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Tadpoles are able to perceive the presence of predators via kairomones and react with changes in behaviour and/or development. However, acute changes in behaviour can also be caused by substances solely released by injured members and hint at the existence of special substances used for intra-species communication in dangerous situations. While receiver tadpoles can benefit from the perception of “damage-released alarm substances” by evading predation, a benefit for the sender, most likely not surviving a predator attack, remains unclear. Furthermore, the identity of damage-released alarm substances as well as their actual release mechanism, remains fairly unknown, thus rendering us unable to classify them as signals or cues. To contribute to their classification in tadpoles, we set up experiments enabling us to investigate behavioural and molecular reactions during a predation event between species with distinct life-history characteristics. We assigned tadpoles forming schools (*Bufo bufo* and *Rana temporaria*) and solitary living tadpoles (*Ranitomeya sirensis*) to three treatments, which reflect typical sections of a predator attack. Tadpoles experienced either: (i) no predator attack (control), (ii) an injury (predator attack) or (iii) damage-released alarm substances by conspecifics (post-predator attack) and were recorded to quantify their response before and after stimulus application. After behavioural trials, tadpoles were euthanized for subsequent RNA-sequencing and differential gene expression analysis in future studies. A holistic interpretation of our current behavioural and future transcriptomic data opens up the possibility to classify damage-released alarm substances. Identification of specific gene sequences (specialized proteins) in species with well documented anti-predator response to damage-released alarm substances, support the hypothesis for a more efficiency-based evolution of alarm substances and active release of specialized substances. Thus, it stands to reason that both sender and receiver gain fitness benefits from this interaction, allowing damage-released alarm substances to be classified as pheromones.

## Preferential processing of chemosensory satiety cues

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Based on several animal and human data demonstrating effects of diet on body odor quality, the current study examines neural responses to satiety- and fasting related human volatiles. One aim is to detect the putative social communicative function of these volatile signals. Axillary sweat was sampled with cotton pads from 10 individuals after 12 hours of fasting, and after consumption of a standard breakfast (identical 2-hour sampling duration in each within-subject condition). Pure cotton pads served as control. The chemosensory stimuli were presented to another 20 participants (via a constant-flow olfactometer). EEG was recorded (61 electrodes), amplitudes and latencies of the chemosensory event-related potential (CSERP; P1, N1, P2, P3) were analyzed and Low Resolution Electromagnetic Tomography Analysis (LORETA) was conducted. The amplitudes of all positive CSERP components differed more strongly from cotton in response to chemosensory satiety cues compared to fasting cues (P1:  $p = .023$ , P2:  $p = .083$ , P3:  $p = .031$ ), paralleled by activity within the middle frontal and temporal gyrus. Latencies were unaffected by the chemosensory stimuli (all  $ps \geq .286$ ). Neither chemosensory stimulus was detected more often than chance (all  $ps \geq .968$ ). The pattern of neural processing suggests satiety sweat to contain rich and complex social information about another individual which is readily attended to and processed as important social information in the human brain. The participants' low detection performance indicates that this information is processed without conscious evaluation.



## Association between self-reported and third party reported attractiveness in body odour

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Human mate choice is driven by various characteristics including body odour attractiveness. The estimation of one's own attractiveness modulates one's self-perceived mate value and consequently mate choice. Previous studies tested self-assessment of various characteristics such as facial attractiveness, but similar data on body odour attractiveness is missing. In our study, we focused on the association between self-reported and third party reported body odour attractiveness in both women and men. We included data from nine different studies. In total, the body odour samples were provided by 277 men and 249 women. Participants also reported the estimation of their body odour attractiveness, body hair density and intensity of perspiration on a 7-point Likert scale. Each sample was evaluated at least by 14 raters (105 men and 965 women in total) for attractiveness. The results of our study showed a significant correlation between self-reported and third-party reported body odour attractiveness in women ( $\tau = 0.16$ ,  $p = 0.001$ ), but not in men ( $\tau = 0.07$ ,  $p = 0.1$ ). Interestingly, the self-reported attractiveness was negatively modulated by the self-reported intensity of perspiration, but it did not influence the third-party ratings of the attractiveness. Both men and women had also a tendency to underestimate their body odour attractiveness (est. = -0.89,  $p = 0.003$ ). This could be due to the better olfactory abilities in women and/or a higher social pressure on the women's attractiveness in physical characteristics.

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## Maternal body odor helps the development of rapid face categorization in the human infant brain

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In humans, maternal body odor conveys probably the most important olfactory cues in the first months of life. Recently, it has been shown that, in the 4-month-old infant brain, the ability to rapidly (i.e., stimulus duration: 167 ms) categorize faces (i.e., discriminate faces from other objects and generalize this discrimination across individual faces) is boosted in terms of an increased face-selective response in presence of the mother's body scent. Interestingly, this maternal odor effect on neural face categorization declines progressively as the latter develops. In the present study, we will investigate how the maternal odor effect on rapid face categorization depends on the difficulty of this task for the sole visual system by measuring a face-selective neural response using scalp electroencephalography (EEG) in 4- and 15-month-olds. The main hypothesis is that the odor effect will disappear at 4 months and reappear at 15 months when face categorization is made less or more challenging, respectively. At 4 months of age, we will compare the face-selective response for two kinds of face stimuli: variable natural images vs. homogeneously edited images without their background. We predict that the face-selective response will increase for edited images, thereby reducing the odor effect. At 15 months of age, we will compare the response for two stimulus durations: 167 vs. 83 ms. At this age, we predict that the face-selective response will decrease for the shortest duration, thereby increasing the odor effect. If our hypothesis is verified, it will support that maternal body odor, as a powerful cue in infancy, is ideally suited to disambiguate visual information and favor the visual perception of conspecifics. More generally, it will suggest that face categorization, and probably perceptual categorization in general, relies on multisensory inputs when unisensory perception is not effective on its own.

## Perianal secretion marking behaviour of the European mink in the face of visual and olfactory signals by conspecifics and predators

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Several solitary and twilight meso-carnivores rely primarily on chemical communication to detect signals from the environment. This communication allows them to face various challenges such as competition for resources and access to breeding pairs or predation by larger carnivores. Our objective was to determine variations in the duration of perianal secretion marking behaviour (PSMB) of 24 European minks in simulated presence of a conspecific (visual signal) and of two potential predators (olfactory signal), terrestrial (dog) and aerial (Eurasian eagle owl). Individual focal sampling with a one-zero time recording was carried out to behavioural data collection. Our statistical model showed that females and subadults devoted less time than males and adults to PSMB, probably due to a more cautious character of females and social inexperience of younger individuals. The increase in PSMB only in the case of males and adults suggests a motivation for intrasexual competition. These individuals showed a high increase on PSMB when they were exposed to the terrestrial predator odor, probably due to detection of volatile substances from carnivore faeces. The combination of cues from conspecific and terrestrial predators showed that minks prioritize a decrease in PSMB as anti-predator response to only the terrestrial predator, omitting conspecific presence. This study highlights the maintenance of the innate response to a predator such as the dog even though minks were born in captivity and were never exposed to predators. However, aerial predators such as an owl can hinder the success in individual reintroductions into the natural habitat, since we do not know if the anti-predatory response to raptors is learned or has been lost due to keeping several generations in captivity. In any case, anti-predatory training against raptors can be useful to reduce mortality in future reintroductions.

## Analytical and behavioural characterization of body odour constituents in the songbird *Taeniopygia guttata*

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In contrast to the assumption that birds are anosmic or at best hyposmic, research during the last decades showed that birds have a fully functional olfactory system (Caro & Balthazart, 2010). It is now generally recognized that birds use olfactory cues in navigation and foraging, and also in social contexts, e.g. in the contexts of reproduction, kin recognition and predator avoidance. In the zebra finch, *Taeniopygia guttata*, evidence has been obtained that volatile substances contribute to the discrimination of kin and non-kin and of own and conspecific eggs, to nest preferences, and to the typical begging behaviour of chicks (Krause et al., 2018). In 2017, Caspers and colleagues found out that freshly hatched chicks begged longer to the parental odour than to the odour of unfamiliar adults. However, the substances involved in eliciting a begging response are thus far unknown. We aim to elucidate these substances. To achieve this aim, we will firstly characterize the volatile composition of the body odour of zebra finches. Different odour sources, such as the whole body odour, the odour of the uropygial gland secretion and the odour of feathers will be investigated and compared with regard to their overall volatile composition and behavioural activity. Analytical methods used are gas chromatography-mass spectrometry and gas chromatography-olfactometry, the combination of which allows distinguishing between volatile and odour-active compounds. Here, we want to present the different methods and steps that we aim to apply to comprehensively characterize zebra finch odour and identify the compounds that elicit a begging behaviour in zebra finch hatchlings.

## A cases of atypical sexual attractiveness in a spayed male of domestic dog - a case study

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In domestic dogs specific play of hormones including estrogens and progesterone is believed to be responsible for the female attractiveness to the males. In this report we are presenting a case of atypical attractiveness of the spayed male dog to other intact males. In the reported case 5 year old spayed male Border collie has been found as extremely attractive to various males, which presented high levels of sexual arousal, with intensive sniffing, licking the area of prepuce, specific vocalization, increased salivation and, finally, mating attempts. Clinical examination of the “case male” revealed a lack of testes in the scrotum and abdominal cavity confirmed by USG. Laboratory tests indicated basal levels of estradiol, testosterone, and progesterone, and cytogenetic and molecular analysis confirmed its gender. Lack of estrogenization was also confirmed by cytological examination of the smears collected from prepuce mucosa. Chemical analysis (GC/MS) of the urine indicated a huge similarity to the profile obtained from a bitches in estrus, with an elevated level of acetophenone, which has been previously postulated in the literature as being a characteristic for the estrus phase in female domestic dogs. As a hypothesis requiring verification, we propose the idea of involvement of other hormones in the creation of incidental attractiveness or increased production of compounds responsible for attractiveness resulting from metabolic events unrelated to reproductive processes. More detailed study of similar cases could be helpful in shedding new light on the process of creation of sexual attraction in the domestic dog.

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