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ABSTRACTS

Social interaction and acoustic communication male and female mice: Beyond the gender difference

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Adapted social behavior allows both individual and collective well being. At the individual level, it is a hallmark of health. Indeed, virtually all mental health disorders are associated with social deficit. We are interested in understanding the behavioral, neural and neurochemical bases of social cognition and communication using mouse models. Here, we will review our recent data showing the relationship between multi-

ple behavioral markers, including ultrasonic vocalizations during dyadic social interaction. We will see the role played by the environment of life, by the context in which interactions take place in healthy individuals and by gender. Together, the data presented will offer novel insight about social life of rodents, its diversity and richness.

Towards a real-time audiovisual tracking system at millimeter precision

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Ultrasonic vocalizations (USVs) fulfill an important role in communication and navigation in many species. Because of their social and affective significance, rodent USVs are increasingly used as a behavioral measure in neurodevelopmental and neurolinguistic research. Reliably attributing USVs to their emitter during close interactions has emerged as a difficult, key challenge. If addressed, all subsequent analyses gain substantial confidence. In this presentation I will detail our recently

developed tracking systems, HyVL and UltraEx, which are the first to achieve millimeter precision (3-5 mm), and thus allow a high degree of reliably assigned USVs. UltraEx also support live tracking and thus provides a direct output of tracked data to the user. It is easy to setup and use, relying on open source software and a single acoustic camera. I will showcase some data analyzed with this system from mouse courtship interactions as well as mother-pup interactions.

USV source identification for investigating neurobiology of social behavior

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Rats and mice are important animal models in studying the neurobiological aspects of social behavior. While they emit numerous ultrasound vocalizations (USVs) during social interactions, little has been explored on their neural correlates compared to bodily motion. This is partly due to the difficulty in distinguishing a source (emitter) of each USV during social interactions involving multiple animals. In our previous study, in which we identified USV source through wireless recording of laryngeal electromyograms (EMGs) in interacting rats, we showed that many neurons in amygdala responded to USVs, and their activities depended on the source (self/other). Considering that the amygdala is postulated to be one of the most important brain regions for social behavior, these results underscore the importance of USV recording and source identification in the analysis

of neural dynamics during social behavior. However, the identification methods using wireless EMGs recording are invasive and technically challenging. Therefore, for simpler source identification, we recently developed an acoustic camera system for USVs called USVCAM. It can localize USVs within a camera view using a compact microphone array attached to the camera. The hardware design allows for applications in social interactions in conventional home cages (and possibly other various apparatuses), as well as fast processing of sound localization. Using USVCAM, we found novel and complex uses of USVs in female mice of ICR strain under a resident-intruder paradigm. Such a simple USV source identification system will greatly facilitate future studies investigating neurobiology of social behaviors using rats and mice.

Modifications in the emission of 50-kHz USVs in rats repeatedly treated with amphetamine and dopamine receptor antagonists: new insights on the role of dopamine transmission in ultrasonic calling behavior

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Earlier investigations have demonstrated that the pharmacological antagonism of D1-like and/or D2-like dopamine receptors suppresses the emission of 50-kHz USVs elicited in rats by the acute administration of psychostimulant drugs with addictive properties. Conversely, no information is currently available on how dopamine D1-like and/or D2-like receptors modulate the emission of 50-kHz USVs in rats repeatedly treated with psychostimulant drugs. To address this issue, we evaluated the emission of 50-kHz USVs in rats that received the repeated administration (×5 on alternate days) of amphetamine (1 mg/kg, i.p.), alone or together with: i) SCH 23390 (0.1–1 mg/kg, s.c.), a dopamine D1 receptor antagonist, ii) raclopride (0.3–1 mg/kg, s.c.), a selective dopamine D2 receptor antagonist, or iii) a combination of SCH 23390 (0.1 mg/kg, s.c.) and raclopride (0.3 mg/kg, s.c.). Rats' calling behavior was evaluated

during pharmacological treatment, as well as 7 days after treatment discontinuation in response to amphetamine-paired environmental cues and amphetamine challenge. Amphetamine-treated rats displayed a sensitized emission of 50-kHz USVs in response to repeated drug administration, as well as an increased emission of 50-kHz USVs in response to the presentation of amphetamine-paired cues and amphetamine challenge. These effects were either suppressed or partially attenuated in rats repeatedly treated with amphetamine preceded by SCH 23390, raclopride, or their combination, depending on the specific antagonist(s) administered and doses thereof. Taken together, the present results may shed further light on how dopamine transmission modulates the emission of 50-kHz USVs in rats treated with psychoactive drugs.

Pups' ultrasound vocalizations in the neurodevelopmental model of schizophrenia

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Schizophrenia is typically diagnosed during late adolescence or early adulthood, but subtle deficits in communication and sociability are often evident from early infancy. Despite this, socio-communicative deficits are poorly investigated in animals at an early stage of development in schizophrenia-like models. These impairments can be experimentally modeled using rodents' ultrasonic vocalizations (USVs). Specifically, USVs emitted by pups separated from mothers/nest serve as a useful tool to reveal reduced attachment with the mother. Using a neurodevelopmental model of schizophrenia based on the prenatal injection of methylazoxymethanol acetate (MAM; 22 mg/kg; embryonic day 17), we studied early communicative behavior by maternal separation-induced USVs in pups. Rat pups of both sexes were examined in the three experimental sets. Firstly, we assessed the pups' sensitivity to famil-

iar and unfamiliar stimuli. Secondly, we evaluated the course of the vocal development. Finally, we investigated the pups' sensitivity to the phenomenon known as maternal potentiation. The general conclusion is that prenatal exposure to MAM results in reduced ultrasonic vocalization in offspring. Poorer vocalization manifests in a lower number of 40 kHz calls, reduced bandwidth, and greater distribution of flat USVs. These changes can be detected at an early stage of infant life, and the degree of impairments may depend on the day of life and/or stress factors. The present results demonstrate that qualitative along with quantitative analyses of neonatal vocalizations is a useful tool for assessing early socio-communicative deficits in schizophrenia models.

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Rapid appearance of negative emotion during oral fentanyl self-administration: Insights from DeepSqueak

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The global opioid epidemic has been fueled in recent years by the widespread availability of cheap and potent fentanyl, which produces an intense and rapid euphoria followed by severe withdrawal and emotional distress. Here, we developed a new preclinical model of volitional oral fentanyl seeking in outbred male and female rats to study the motivational and emotional aspects of synthetic opioid use. We also recently upgraded our open-source software, DeepSqueak v3, to utilize variational autoencoder embeddings to improve unsupervised classification of ultrasonic vocalizations (USVs), which are innate expressions of current emotional state in rats. Using a traditional two-lever operant procedure, rats learned to self-administer oral fentanyl, escalated fentanyl intake across sessions, and reinstated responding to conditioned cues after extinction. Oral SA also revealed individual and sex differences that are essential to studying substance use risk

propensity. During a behavioral economics task, rats exhibited inelastic demand curves and maintained stable fentanyl intake across a wide range of doses. Oral fentanyl self-administration was also clearly patterned, with distinct “loading” and “maintenance” phases of responding. Animals produced positive-affective 50 kHz USVs during loading, then transitioned quickly to negative-affective 22 kHz calls despite ongoing maintenance of oral fentanyl seeking. The affective opponent process theory of addiction postulates that addiction is driven by both the initial pleasurable effects of drugs and the subsequent negative affective state that slowly emerges as a result of repeated drug use, leading to a cycle of drug-seeking to alleviate negative affect. Our results indicate this progression from positive to negative reinforcement occurs earlier than previously thought, even within an active drug taking session, thereby revealing a “within-session” opponent process.

Context-specificities of ultrasonic vocalization characteristics in laboratory mice

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The diversity of social communication systems across taxa underlines the need of distinctive investigations in each of them to understand their evolutionary relationships. Rodents and more specifically mice are organisms used as models for human disorders affecting communication, such as autism. Therefore, understanding their social communication system is fundamental to control the biological framework in which the mechanisms of the disorders are studied. For this purpose, we focused on the spontaneous behaviors of pairs of familiar mice living undisturbed for several days. We aimed at identifying contexts of emission of specific vocalization characteristics by analyzing synchronously

the social and vocal behaviors of pairs of adult C57BL/6J female mice. In these conditions, a sample of acoustic characteristics were context-specific. We also noted an important inter-pair diversity, that could be linked to peculiarities in the behavioral organization of each pair. Altogether, our approach of spontaneous emission of ultrasonic vocalizations shed light on some fundamentals of this mode of communication in mice. It suggests that ultrasonic vocalizations represent a robust complementary readout to fine-tune social behavior description and interpretation. It will benefit from future investigations in strains closer to wild mice, as well as from the identification of the vocal emitter.

Adult rat ultrasonic vocalizations: useful indices of positive or negative affect?

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To misquote George Orwell's novel *Animal Farm*: "Adult rat 50-kHz calls good, 22-kHz calls bad". The main aim of my presentation is to critically examine and refine this notion, by reviewing a broad range of studies that have used drugs and behaviorally-relevant stimuli. I will argue, first, that while 22-kHz calls may indicate negative affect under some conditions, this is by no means a general finding. Attributing emotional significance to 50-kHz calls, in turn, is complicated by their marked spectrographic heterogeneity. Unfortunately, there is no consensus as to how 50-kHz calls should be categorized (at least among human observers). We have proposed more than a dozen 50-kHz call subtypes, exploring how the relative prevalence of these many call subtypes changes after drug challenge and in some behavioral contexts. This granular-and laborious-ap-

proach has revealed that play behavior and several euphorogenic drugs preferentially promote a particular subset of frequency-modulated 50-kHz calls (trill subtype), at the expense of constant-frequency (flat) calls, whereas the opposite shift can be seen during acute morphine withdrawal. However, more recent findings, with other aversive drug states and with reward anticipation, suggest that a yet more nuanced view of 50-kHz calls is needed if they are to serve as a readout of rat "affect". Lastly, following our recent report that rats maintain months-long inter-individual differences in call subtype prevalence, I will discuss the possibility of a rat "USV personality trait".

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Improving translational research models for human mental disorders: Deciphering socio-affective communication through ultrasonic vocalizations in rats

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There is a need for improved animal models to advance brain sciences. This includes animal models of human mental disorders and their treatment. Because the diagnostic criteria for mental disorders are purely behaviorally defined, the validity of rodent models strongly relies on their behavioral phenotype. For this reason, deep and longitudinal behavioral phenotyping constitutes the principal component for current translational research, yet this strategy depends on sensitive behavioral assays with high relevance to each diagnostic symptom category. Rats display a rich social behavior repertoire. This includes socio-affective communication through the emission of calls above the human hearing threshold of ~20 kHz, so-called ultra-

sonic vocalizations (USV). Typically, three main types of USV are distinguished: (A) Isolation-induced 40-kHz USV in pups, as well as (B) aversive 22-kHz USV and (C) appetitive 50-kHz USV in juvenile and adult rats. Specifically, 22-kHz USV occur during and in anticipation of aversive events, e.g. predator exposure or fear learning, while 50-kHz USV occur during and in anticipation of appetitive events, e.g. social play or in response to psychostimulants, most notably amphetamine. In my talk, I will provide an overview on socio-affective communication in rats and show how studying 22-kHz and 50-kHz USV in sender and receiver can be used to assess behavioral alterations with relevance to affective and neurodevelopmental disorders.

Functions of 22 kHz ultrasonic vocalizations in rats resulting from aversive arousal

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It has been well established that activity of the mesolimbic tegmental cholinergic system brings about emission of aversive 22 kHz vocalizations in rats. This emission signals negative (unpleasant) emotional state of the animal. The emission may be caused by natural threat or other danger or by direct pharmacological activation of this system. While emission of 50 kHz ultrasonic vocalization signalling opposite, appetitive state, has been analyzed in detail and subdivided into many

subcategories, the emission of 22 kHz has not been thoroughly analyzed and categorized. This presentation will describe the brain aversive arousal system and its mode of functioning, basic types of 22 kHz vocalizations induced in different situations, and the presumed communicative role of these calls. Emission of 22 kHz calls presents a classical form of ethotransmission, i.e., a rapid vocal signalling system in mammalian brain, and its biological role will be discussed.

Ultrasonic vocalizations as a model of social communication in neurodevelopmental conditions

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Ultrasonic vocalizations (USVs) are produced by rodents in different social contexts across the lifespan. USVs in rodents have been used as a model for understanding human communication. In murine models of neurodevelopmental conditions with a genetic basis, like autism spectrum disorders (ASD), the study of USVs has provided the unique opportunity to evaluate social communication and interaction in ethologically-appropriate contexts. We evaluated ultrasonic vocalizations'

data collected in murine models of ASD in different experimental settings, either in the neonatal phase or in adulthood. Both quantitative (calling rates) and qualitative (range and shape of the vocal repertoire) abnormalities have been evidenced. Studying murine USVs in neurodevelopmental disorders may improve our understanding of the speech deficits observed in humans, especially in minimally verbal children with ASD.

Exploring generalizability in ultrasonic vocalization detection and classification methods

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House mice communicate through ultrasonic vocalizations (USVs) exceeding the range of human hearing (>20 kHz). Automated methods have been developed to detect and classify these USVs. However, due to limited publicly available labeled data and the labor-intensive nature of manual data labeling, researchers frequently employ pre-existing “out-of-the-box” tools across various laboratory settings (e.g., different contexts, different species, or different mice generations). This practice introduces concerns regarding model performance and generalizability, which remain relatively unexplored in USVs research. This study aims to comprehensively evaluate USV detection and classification methodologies while introducing an innovative approach. We conducted a comparison of four detection tools,

DeepSqueak detector, MUPET, USVSEG, and automated mouse ultrasound detector (A-MUD), all assessed under an “out-of-the-box” framework. A-MUD outperformed the other methods in terms of true positive rates and false detection rates. For automated USV classification, we introduce BootSnap – a supervised classification tool leveraging bootstrapping on gammatone spectrograms and convolutional neural network algorithms, enhanced by Snapshot ensemble learning. BootSnap successfully categorized USVs into 12 distinct types, identifying a novel category of false positives useful for USV detection refinement. BootSnap outperformed the pretrained and retrained state-of-the-art tool, and thus it is more generalizable.

Providing better translational insights into substance use disorders using ultrasonic vocalizations

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Emotional states are powerful modifiers of behavioral response and are becoming ever more ensconced in neuroscience research. The dysregulation of emotional states is a key facet of multiple psychopathologies, making measures of emotional state of interest in preclinical models of mood disorders, anxiety disorders, drug addiction, eating disorders, or even autism. In Particular, substance use disorders are often accompanied by dysregulated emotional processing that can act as powerful drive states for future substance misuse and drug

relapse. The present talk will review the juxtaposing roles of positive and negative drive states in substance use disorder and how they relate to specific aspects of substance use disorders. We will also discuss the invaluable contribution of rat ultrasonic vocalizations (USVs) as a preclinical analog for emotional processing in humans and detail some of the work by our lab and others showing the time course for changes in affective responses during substance misuse.

Are we missing something? Exploring aversive calls in the 44-kHz frequency band

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In literature, adult rat ultrasonic vocalizations (USV) are often divided into three main groups depending on their frequency band and call duration. These are i. long (>300 ms), monotonous, low-pitched (<32 kHz) calls named long 22-kHz USV; ii. short (<300 ms) and monotonous, low-pitched 22-kHz USV referred to as short 22-kHz USV; and iii. short (<150 ms) and usually frequency-modulated, high-pitched (35–80 kHz) 50-kHz USV. We have observed and described a repeating appearance of long (>150 ms) and monotonous but high-pitched (35–50 kHz) USV. These USV were emitted during prolonged fear conditioning in both Wistar rats and spontaneously hypertensive rats. In contour-based and frequency-time-based clustering analyses of these calls, they formed distinct clusters from other previously established USV groups. We named these USV “44-kHz USV”. They are, most likely, aversive calls, since they are emitted during prolonged fear conditioning.

Also, many 44-kHz USV had a compound structure and contained elements of 22-kHz USV. Finally, the response to 44-kHz USV playback to rats was more akin to that of aversive 22-kHz USV than to appetitive 50-kHz USV, e.g., regarding changes in heart rate. The results have been published in: <https://www.biorxiv.org/content/10.1101/2023.04.06.535936v1> (preprint);

<https://elifesciences.org/reviewed-preprints/88810> (reviewed preprint).

Recently 44-kHz USV were shown by others in a similar experimental setup. Gonzalez-Palomares et al. (Scientific Reports, 13: 11173, 2023) reported these USV during prolonged odor fear conditioning in Long Evans rats. 44-kHz USV appeared especially during 8–10 trials – in contrast to the 1–3 trials (similar to our findings). Altogether, these results suggest that major USV groups along with USV cladistics may require redefinition.

Ultrasonic vocalizations in mouse models for autism spectrum disorder: A link to synaptic dynamics

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Delayed or absent verbal speech in humans could be a hint depicting communication deficits related to various disorders, including autism spectrum disorder (ASD). However, unlike in humans, testing communication deficits in mice is a challenging task, as means of communication in this species is different than that of humans. Mice, instead, communicate using olfactory social signals and emitting ultrasonic vocalizations (USV) in various situations, depending on age, environment, and genetic factors. Measuring USV at different stages of development and experimental contexts is therefore a useful tool to bring about the communication deficits in mouse models. In fact, alterations in ultrasonic communication have been reported in several mouse models for neurodevelopmental disorders, offering reasonable face validity to diagnostic symptoms of ASD. Typically mice emit three distinct types of USV. Neonatal mice, when isolated from the mother and littermates, emit vocalizations that follow a clear ontogenetic profile, peaking at around the eighth day after birth and decreasing to zero levels at around two weeks of age. Juvenile mice as well emit USV during social investiga-

tion of a conspecific. These USV appear to be specific to the social interaction, as the USV production was found to be significantly correlated with the investigation responses of the mice. In adulthood, USV production can occur during female-female interactions, or during male-female interactions such as in mating behavior. The latter type, possibly inducing receptive behavior in females, are emitted by the male during mating, as well as if the male is exposed to female urine.

During my talk I will first give a very brief introduction on the abovementioned wide repertoire of mouse vocalizations, emphasizing the communicative function of these. To give insights into their significance in neurodevelopmental disorders, I will provide examples of USV alterations in mouse models for neurodevelopmental disorders and their link to social behavior, specifically in mice lacking proteins that act as mediators of dendritic spine formation. This will come with a comprehensive focus on “social communication” category of ASD diagnostic symptom throughout development and under different social contexts to establish a translational link across species.

Machine learning on populations

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Nowadays, we witness a great expansion of the volume of data collected during scientific research; not only the volume of data increases, but also the complexity of individual observations. This is not only a challenge for modelling, but also for the interpretation of the results, which is an essential milestone towards building an abstract foundation for understanding the

given phenomenon, which will always be the ultimate aim. In my talk, I will sketch the idea of atomisation, an approach focused on non-unique separation of independent aspects of the data, and how it can provide a common ground for machine- and human-derived abstractions, utilizing USVs as an example application.

Exploring ultrasonic vocalization's role in reward-related memory and goal-directed behavior

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Ultrasonic vocalizations in rodents serve as well-established indicators of motivational and emotional states. In the experiment, we used the rewarding effects of amphetamine, which induces 50-kHz USV in rats, to study spatial memory consolidation processes. We divided the rats into Recent and Remote groups. We used CatFish methods to analyze neural activity, and imaging neural activity in two separate testing sessions. We analyzed ultrasound emission during six test days. Amphetamine was administrated in one of the four corners of the cage. We demonstrated a reduction in global neuronal activity in the remote group after 14 days of memory maturation and observed the phenomenon of neuronal specification, where particular populations of neurons were activated in both the first and second test sessions. No differences in ultrasound emissions between the Recent and Remote groups during the reward-seeking

task were detected. Correlational analyses of the ultrasound emitted showed that vocalization emitted during the first test session was associated with hippocampal CA1 activity. On the other hand, examining the relationship between training sessions, we observed the relationship of 50-kHz USV with the infralimbic cortex and retrosplenial cortex activity. The gradation and stabilization of USV emission during training and test sessions were observed. A negative correlation between 50-kHz USV emission and conditioned corner visitation was observed. Notably, strong emotional processing post-amphetamine administration reduced correctness values, suggesting that USVs are independent of memory consolidation. These findings provide key insights into the neural and behavioral mechanisms of spatial memory consolidation.

Methods applied in acoustic signal analysis. Which is worth using?

Paweł M. Boguszewski

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The study of audible and ultrasonic (USV) vocal communication in animals is an emerging and evolving field of science. Its primary goals revolve around differentiating meaningful signals and evaluating the content of these communications from an ethological perspective. Researchers employ a range of methods and approaches to investigate auditory signals and that includes several crucial steps: 1) signal recording: collection, pre-processing, filtering and noise reduction; 2) acoustic analysis: amplitude envelopes, frequency spectrograms, pitch detection, temporal analysis, wavelet transform

and 3) pattern recognition and classification: manual detection, principal component analysis and machine learning techniques.

Those methods should be complemented by a suite of techniques, including behavioral observations with detailed ethograms, playback experiments, cross-strains and cross-species comparative studies as well as neurobiological studies for understanding the neural mechanisms involved in vocal production and perception. In my lecture I will endeavor to provide an overview of this methodological landscape.

Social buffering during fear memory extinction

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In social species, proper social interactions are one of the key factors necessary for survival and well-being of an individual. It has been shown that the presence of a partner reduces stress levels in stressed individuals which facilitates recovery from trauma. This phenomenon, discovered in humans and several others social species, is called social buffering. We are interested in social buffering especially in the context of exposure-based psychotherapy, widely used to treat pathological fear such as phobias and post-traumatic stress disorder (PTSD). The transient and context-dependent character is a major drawback of such therapies. The main goal of our research was to check if social support can be beneficial for such therapies. To address this issue we have designed a protocol of social buffering

during fear memory extinction, a widely used animal model of humans fear psychotherapies. We observed that fear conditioned but not extinguished rats tested in the presence of an extinguished partner show decreased level of freezing in comparison with rats tested alone. During the “together” test we recorded mainly ultrasonic vocalization with frequency of about 55kHz while solitary testing was associated with 22 kHz vocalizations. This effect depended on partners emotional status, familiarity, and strain sameness of tested animals. Finally, social buffering effect on fear response was transient and effective only in the presence of the partner but not when rat was tested alone the next day, which suggests that social buffering in our model is not a form of extinction learning.

Quantification and synchronization of vocalizations with live mouse tracker

Fabrice de Chaumont, Benoit Forget

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During this talk, 4 topics will be covered. First, the quantification and synchronization of vocalizations with live mouse tracker. We will display a preliminary work of 3 new kinds of vocalizations that we are trying to decipher. Second, we will show how the real time capabilities of LMT are ready to use an active beamforming algorithm, and how live mouse tracker can play a role in the enhancement of the prediction of the identity of the vocalization emitter. Then we will show for the

first time in a conference our last fast-learning smart environments called the Live Mouse Tracker Blocks, and open discussion on how to use this kind of environment to use close loop playback and live voc classification with mice and rats. Finally, we will show the first alpha implementation of our Shazam-like algorithm for USVs, designed to help the everyday investigators of the vocalizations' meaning.

Exploring the emotional side of ticklishness: Insights from insular neurons

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Since Darwin described diverse biological aspects of emotions, understanding internal states has become one of the major challenges with lots of debate on emotions definition and their expression across species. Yet, brain mechanisms underlying internal states that drive evolutionary conserved behaviours remain elusive. Ticklishness is a peculiar sensation seen in social species from rodents to humans. When tickled, rats emit appetitive 50 kHz ultrasonic vocalizations, in addition to pronounced chasing behaviour, emphasizing the rewarding nature of tickling. Previous work showed the activation of the trunk somatosensory cortex (S1) in response to tickling. Microstimulation in S1 deep layers equally evokes vocalizations (Ishiyama & Brecht, 2016). It is, however, unknown how the tickle tactile sensation is coupled to a positive emotional state. In this study, we focus on the insula being a central hub for emotional processing and receiving many inputs from sensory areas. *In vivo* freely-moving extracellular recordings revealed activation of insular neurons during

tickling and no-contact play but less so during gentle touch, indicating insular response to playful context. Hierarchical clustering resulted in units with differential response patterns to tickling, which were consistent across different playful events. Spike waveform analysis suggested distinct physiological characteristics of the play-activated cluster. Pharmacological manipulation of the global insula including inhibition and disinhibition showed no effect on play behavior assessed by call rate, chasing duration and jump rate. Anterograde tracing showed abundant projections from the granular/dysgranular insula to areas expressing cFOS following tickling such as the amygdala, accumbens, and thalamus. Our preliminary results suggest that the insula, through anatomical, functional and physiological correlates, might be implicated in the processing of tickle-induced playful emotions. We will further continue investigating potential circuits involved in the modulation of ticklish emotional response.

The role of ultrasonic vocalizations in translational neuroscience

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Positive and negative emotional states in rats can be studied by using ultrasonic vocalizations (USVs). Positive emotional states are indexed by 50 kHz hedonic USVs, and negative affect is indexed by 22 kHz aversive calls. We recorded EEG from the MPFC of rats and showed that positive affect was associated with active wake EEG (greater alpha-delta ratio), and aversive states with groggy wake EEG (high delta low alpha). Electrical stimulation of the MPFC at the alpha frequency induced hedonic calls and increased synaptic strength Long-term potentiation (LTP), and the converse was seen with delta frequency stimulation (Long-term depression (LTD)). The final common output pathway for vocalizations, the periaqueductal gray (PAG), shows a motor-evoked potential that is time locked to the alpha

(hedonic) and delta (aversive) motor-evoked potential. Time series studies showed that the alpha delta ratio could predict the onset of aversive calls and could be prevented by closed loop alpha stimulation. At the neuronal level, alpha power is driven by excitatory glutamate activity and delta power by the lack of excitatory input, and that excitatory but not inhibitory neurons drive these rhythms. In humans, positive affect is also associated with higher frontal alpha/delta ratios, and negative affect with the converse. In conclusion, the synchronization of alpha/delta oscillations coordinates emotional behavior, and the switch between active wake/positive affect and groggy wake induced negative affect appears to be controlled by a LTP/LTD mechanism in the medial prefrontal cortex.



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