ABSTRACT BOOK

Systems- **IN** - Action

Advances in brain circuit research in systems and behavior

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1. Shining light on higher visual cortical activity across visual tasks.

Virginie Oberto, Sakir Kaan Cetindag, Mihaela Gerova, Dylan Myers-Joseph, Asli Ayaz, Vincent Bonin.
Neuro-Electronics Research Flanders (NERF), KU Leuven ,Leuven, Belgium

The brain continuously transforms sensory inputs into meaningful perceptions that guide flexible actions. This relies on specialized regions that extract and prioritize distinct sensory features and task-relevant information, making it essential to engage the appropriate brain areas to produce the correct behavioral output. Using the mouse visual system as a model provides a tractable framework for dissecting how specialized circuits support visually guided tasks. It includes about a dozen higher visual areas (HVAs), each with distinct connectivity and response properties, reminiscent of primate visual streams. Although HVAs are known to participate in visual behaviors, it remains unclear how specialized areas contribute when animals perform distinct tasks requiring selective attention to specific visual features.

To address this question, we trained mice on three visual discrimination tasks based on the location, size, or orientation of stimuli. The animals successfully learned and performed all tasks with comparable accuracy. To examine whether distinct neural circuits are recruited during behavior, we recorded large-scale activity across the dorsal cortex using widefield one-photon Ca^{2+} imaging. During the size discrimination task, trial-by-trial analyses suggested that changes in stimulus size tended to modulate activity primarily within visual cortices. Primary visual cortex (V1) and the more anterolateral higher visual areas: anterolateral (AL), rostrolateral (RL), and lateromedial (LM), showed the strongest and most consistent activation patterns.

To assess the causal contribution of these areas and determine whether increased large-scale activity correlates with performance, we applied mesoscopic patterned-light optogenetic suppression. During the size discrimination task, inhibiting V1 and these HVAs led to noticeable reductions in performance, with the largest effects following suppression of V1 and the anterior visual areas (AL/RL). To examine how task-specific these effects might be, we performed similar perturbations during the location detection task. In that case, overall performance was largely maintained across most HVAs, although reaction times tended to increase.

Overall, these preliminary results suggest a non-uniform involvement of visual areas across different visual behaviors. Building on this, we are now using simultaneous two-photon calcium recordings in multiple HVAs during behavior to uncover the cellular-level mechanisms that shape how visual and contextual information is distributed across areas and how this distribution gives rise to their distinct causal roles across tasks.

2. Behavioral orienting but not novelty activates dopamine neurons.

Kondrakiewicz, **Kacper** [1], Temmerman, Xander [1], Luijten, Sofie [1], De Paep, Len [1], Haesler, Sebastian [1,2]

1 - NeuroElectronics Research Flanders (NERF), Leuven, Belgium

2 - Department of Neurosciences, KU Leuven, Leuven, Belgium

Novel stimuli have a profound impact on brain function and behavior. They evoke attention, arousal and sensory exploration and induce synaptic plasticity in brain regions related to learning and memory. Many of these effects have been attributed to novelty-evoked activation of the dopaminergic midbrain and dopamine (DA) release in major projection targets in the striatum. However, it remains controversial if individual DA neurons respond to novel stimuli. To address this question, we recorded and manipulated DA neurons in mice exposed to novel and familiar odors while monitoring orienting behaviors including exploratory sniffing, facial movements, and pupil dilation. We found that DA neurons were activated by orienting behaviors, rather than by novelty itself. Moreover, their activity was causally involved in generating the orienting responses. Finally, we identified a major input region to the dopaminergic midbrain which couples orienting to DA firing. Activity in the mediodorsal pons (mdPons) correlated tightly with sniffing and facial movements. Consequently, optogenetic stimulation of glutamatergic mdPons neurons induced both orienting and DA firing. Our findings suggest that DA neurons do not respond to novelty per se but are activated through descending glutamatergic projections from the mdPons. This mechanism links salient sensory events to DA release, providing a basis for novelty-evoked exploration and plasticity.

3. The anterior olfactory nucleus is essential for mediating exploratory behaviors induced by novel olfactory stimuli.

De Plus R[1], Broux M[1], Giaffar H[3], Schiltz E[1], Kondrakiewicz K[1], Aydin C[1,4]†, Haesler S [1,2],†
1Neuroelectronics Research Flanders (NERF), Leuven, Belgium
2Department of Neurosciences, KU Leuven, Leuven, Belgium
3Halıcıoğlu Data Science Institute, University of California, San Diego, La Jolla, USA.
4Department of Biomedical Engineering, Bahcesehir University, Istanbul, Turkey.
†Correspondence: cagatay.aydin@nerf.be or sebastian.haesler@nerf.be

When novel stimuli are detected, humans and animals react with orienting and exploratory behaviors driven by sensory input. For example, mice exposed to novel odorants respond with an increase in respiration frequency and pupil dilatation. These spontaneous responses reflect the adaptive value of exploring unfamiliar stimuli in complex environments.

How novel odors are discriminated throughout the early olfactory pathway is poorly understood. We used a spontaneous novelty detection paradigm to study how the olfactory system processes novel and familiar odors. We trained mice for four days while familiarizing them with an odor set. Then, we introduced a novel odor set on the fifth day to compare the influence of novel and familiar odors on behavior and neuronal responses. Our data showed stronger neural responses to novel odors in the Anterior Olfactory Nucleus (AON), compared to the Piriform Cortex (PCx). Hence, we hypothesized that the AON, but not the PCx, is crucially involved in processing stimulus novelty. To verify this, we inhibited these regions with muscimol infusions and observed respiration and pupil diameter during the spontaneous novelty detection paradigm. We found that inhibiting the AON, but not the PCx, reduced sniffing and pupil dilatation in response to novel odors.

Given these results, the subsequent questions remained: a) did AON inhibition selectively influence novelty detection, or odor discrimination in general b) was the effect mediated by AON feedforward or feedback projections. To address these questions, we selectively inhibited the AON neurons that project back to the olfactory bulb (OB) using DREADDs. We found that inhibiting the AON to OB projection caused a reduction in response to novel odors. However, we observed that feedforward projections from the AON to the PCx were also labeled. Therefore, we could not definitively conclude whether the feedback projection from AON to OB or the feedforward projection from AON to PCx is primarily important for discriminating between novel and familiar odors. We are now specifically inhibiting feedforward projections from the AON to the PCx, which will provide insight into the potential involvement of AON to PCx projection in novel odor processing and discrimination.

4. Automated Behavioral Analysis in Parkinsonian Models: From Dyskinesia Clustering to Dopaminergic Modulations Fingerprints.

Alcacer, **Cristina**, Jercog, Pablo
1: Cajal Neuroscience Center (CNC), Spanish National Research Council (CSIC), Madrid, Spain

Dopamine plays a central role in the initiation, sequencing, and suppression of actions. In Parkinson's disease (PD), dopaminergic degeneration leads to bradykinesia and rigidity, while its treatment with L-DOPA often induces abnormal involuntary movements known as L-DOPA-induced dyskinesia (LID). The heterogeneity of LID and related motor complications reflects underlying circuit dysfunctions that remain poorly understood, in part due to limitations in accurate behavioral quantification.

To overcome these limitations, we previously developed a semi-automated pipeline for high-resolution classification of dyskinetic behaviors in freely moving mice (Alcacer et al., Cell Reports 2025). Using head-mounted inertial sensors (IMUs), bottom-up video, and unsupervised behavioral clustering, we identified two dyskinesia subtypes and a novel pathological rotation phenotype. Importantly, calcium imaging revealed that these behavioral motifs were encoded by specific ensembles of hyperactive D1- and D2-spiny projection neurons, many of which also participated in similar normal movements at baseline.

Building on this framework, we aimed to expand the analysis beyond LID to ask how distinct dopaminergic agents - targeting D1 vs. D2 receptors - reshape behavior across dose and lesion state. Using a comprehensive dataset combining open-field video (with mirrored views) and IMUs from intact and 6-OHDA-lesioned mice treated with L-DOPA, SKF-38393, or quinpirole, we aim to extract automatically drug- and dose-specific behavioral fingerprints with sub-second resolution. Our analytical approach combines supervised and unsupervised machine learning (e.g., SVM, XGBoost, A-SOiD) to decode treatment conditions and reveal structure in spontaneous action.

Ultimately, this work seeks to bridge pharmacology, behavior, and circuit function through standardized behavioral metrics with clear analogs in human wearable data. As such, it may lay the foundation for future AI-assisted, personalized treatment strategies in PD, echoing the clinical impact of closed-loop insulin delivery in diabetes.

5. Dorsal Horn Circuit Alterations in Cold Hypersensitivity during Chemotherapy-Induced Peripheral Neuropathy.

Marina Villaro, Félix Viana, Ana Gomis
Instituto de Neurociencias, UMH-CSIC, Sant Joan d'Alacant, Spain

Chemotherapy-induced peripheral neuropathy (CIPN) is a frequent and disabling side effect of neurotoxic anticancer treatments, characterized by peripheral nerve damage. Painful sensory symptoms, particularly cold hypersensitivity, often require dose reduction or treatment interruption, negatively affecting therapeutic outcome and patient survival. The mechanisms underlying CIPN are complex and involve altered function of ion channels in primary sensory neurons. Although these peripheral changes have been widely studied, much less is known about their impact on spinal processing, the first central relay of sensory information. In particular, the identity of spinal interneurons involved in cold signal processing and the possible imbalance between excitatory and inhibitory circuits under pathological conditions remain poorly defined. This project aims to investigate pain processing at the spinal cord level, focusing on dorsal horn circuits involved in the integration and transmission of cold stimuli in a mouse model of oxaliplatin-induced peripheral neuropathy. Given their key role in nociceptive and thermal processing, neuronal activation in laminae I and II of the dorsal horn was specifically analyzed. After behavioral validation of the CIPN model, active dorsal horn neurons were permanently labelled during exposure to different cold stimuli using the TRAP2-iCreERT2-tdTomato mouse line. Neuronal responses to innocuous and noxious cold were compared between oxaliplatin-treated and control mice. Both stimuli induced a significantly higher number of activated dorsal horn neurons in CIPN mice, in line with the enhanced cold sensitivity observed at the behavioral level. To assess the contribution of cold-sensitive ion channels, mice received intraplantar injections of AITC (Trpa1 agonist) or WS12 (Trpm8 agonist). Pharmacological activation revealed that, in control animals, Trpa1 stimulation recruited a larger neuronal population than Trpm8. In contrast, in oxaliplatin-treated mice, both agonists activated comparable numbers of dorsal horn neurons. Notably, comparison between groups showed a significant increase in the recruitment of Trpm8-responsive neurons in CIPN mice. This shift suggests an upregulation of Trpm8-dependent spinal circuits, consistent with the development of cold allodynia. Overall, these findings indicate that CIPN is associated with a reorganization of cold-evoked spinal circuits, highlighting altered recruitment of dorsal horn neurons and a prominent contribution of Trpm8-dependent pathways.

6. Neuromodulatory control of innate defensive behaviours.

Hanson, Laura[1,2], Nuttin, Bram[1,2], Liu, Chen[1,2], Farrow, Karl[1,2]
1; KU Leuven, Leuven, Belgium
2; VIB, Leuven, Belgium

We are born with genetically encoded innate behaviours supported by specialized neural circuits, enabling rapid and reliable responses to ecologically relevant stimuli without prior experience. Although these behaviours rely on stable sensory-to-motor pathways, innate responses are inherently adaptable, adjusting to internal states, environmental context, and one's stage of life. While activation of specific superior colliculus cell types or their output pathways has revealed distinct circuits capable of evoking different behaviours, the mechanisms guiding the brain's selection among these pathways at any given moment remain unclear. We hypothesize that hormonal signaling can modulate information processing within these hard-wired circuits by selectively altering the excitability and gain of specific midbrain pathways, thereby modifying which behaviour is elicited by a specific sensory input. Using behavioural assays, volumetric functional ultrasound imaging, and Neuropixels probe recordings in the superior colliculus and downstream pulvinar, we demonstrate that hormone modulation distinctly alters behavioural outcomes and neural dynamics. Elevated oxytocin levels shift behavioural responses to threatening visual stimuli, decreasing escape behaviours and increasing immobility, coinciding with heightened activity in wide-field neurons in the superior colliculus and the downstream pulvinar neurons. In contrast, elevated corticosterone enhances the frequency and speed of escape responses, accompanied by increased neuronal firing rates and reduced response latency across superficial and deep superior colliculus layers. These findings highlight how hormone-induced modulation of superior colliculus circuits enables dynamic reconfiguration of innate behavioural responses, providing key insights into the neural mechanisms through which internal hormonal states influence sensory processing and adaptive behaviours.

7. Astrocytic Control of Cortical Ensembles Ensures Accurate Sensory-Guided Behavior.

Juliana M. Rosa[1,2], Andrea Misol-Ortiz[1,2].

1. Neuronal Circuits and Behavior Group, National Hospital for Paraplegics, Toledo, Spain.
2. Health Research Institute of Castilla-La Mancha (IDISCAM), Toledo, Spain.

Sensory information is encoded in the brain by neuronal ensembles, coactive groups of neurons whose population dynamics represent specific features of the environment. The precise recruitment of these ensembles is essential to maintain high-fidelity stimulus representations and adaptive, sensory-guided behavior. However, what ultimately determines the activation of the appropriate ensemble for a given sensory event remains unclear. Here, we show that astrocytes critically contribute to this selection process by regulating translaminar information flow within the somatosensory column, thereby ensuring the correct activation of layer 5 output neurons. Using in vivo chemogenetic manipulation of astrocytes in freely moving mice, combined with behavioral assays, microendoscopic calcium imaging, and electrophysiology, we found that peripheral tactile and thermal stimuli engage largely distinct cortical ensembles, with only a small subset of multimodal neurons participating in both. Enhancing astrocyte Ca2+ activity via Gq-DREADDs increased the proportion of multimodal neurons, indicating greater flexibility of single neurons to switch between ensembles in the context of a disrupted astrocytic network. Paradoxically, this increase in multimodality degraded sensory-guided behavior for texture and temperature discrimination, implying that ensembles recruited under altered astrocyte activity become more broadly tuned and less specialized for incoming inputs. These findings identify astrocytes as key circuit elements that constrain neuronal ensemble recruitment, thereby preserving modality-specific processing and supporting accurate sensory-guided behavior.

8. Cooperative cortical and subcortical processing explains differential sensitivity to visual features in mice.

Cetindag, Sakir Kaan, [1,2,3]; Vermaercke Ben, [1,3]; Sans-Dublanc, Arnau [1,2]; Hosseinjany, Shahriar, [1,2]; Farrow, Karl, [1,2,3]; Ayaz, Asli, [1,3]; Bonin, Vincent, [1,2,3]

Neuro-Electronics Research Flanders, Leuven, Belgium; KU Leuven, Department of Biology & Leuven Brain Institute, Leuven, Belgium; VIB, Leuven, Belgium

In natural environments, animals encounter streams of sensory signals that vary widely in reliability, detail, and behavioral relevance. Although several parallel pathways can drive motor responses—even independently—most ethologically important behaviors rely on integrating information across these circuits to produce robust, rapid, and context-appropriate actions. To examine whether distinct pathways preferentially support the detection of specific visual features, we trained mice in a visual detection task that varied stimulus contrast, speed, and spatial frequency, and combined mesoscopic optogenetic suppression of cortical activity in V1 and higher visual areas (HVAs) with chemogenetic inhibition of the superior colliculus (SC). Using spatiotemporally filtered noise stimuli, we show that mice exhibit strong differences in sensitivity to rapid motion with coarse spatial structure versus slow motion with fine spatial detail. Near perceptual threshold, detection of both stimulus classes required activity in V1 and HVAs, consistent with a general cortical dependence when sensory evidence is weak. However, when stimuli were presented at suprathreshold contrast, a distinct division of labor emerged. Detection of slow, high-spatial-frequency stimuli remained almost entirely dependent on cortical processing, whereas detection of rapid, low-spatial-frequency motion was preserved during cortical suppression but abolished when SC activity was silenced. Joint cortical–SC inhibition eliminated detection of both stimulus types, revealing cooperative contributions of the two pathways and identifying the SC as the primary alternative route supporting high-speed motion detection when cortical activity is reduced. Together, these findings show how perceptual decisions arise through coordinated interactions between cortical and subcortical pathways. More generally, the study provides a framework for understanding how parallel neural pathways shape adaptive behavior. By revealing how the brain flexibly engages distributed circuits according to the features and reliability of incoming signals, the work identifies an organizational principle that likely extends across sensory systems.

9. Sex-Dependent Modulation of Emotional and Cognitive Processes by Prefrontal CB1 Receptors.

Ceprian M[1-2], Egaña-Huguet J[1-2], Torres X[3], Godoy L[4-5], Godoy A[1-2], Aranguren-Alberdi A[1-2], Aguirrebengoa O[1-2], Sangroniz-Beltran L[1-2], Reyes-Velasques P[1-2], Torres-Maldonado P[1-2], Santas-Martín JA[1], Ramos-Miguel A[1-6], Tønnesen J[7], Mato S[1-2-6], Soria-Gomez E[1-2-8]

1.University of the Basque Country UPV/EHU; 2. Achucarro Basque Center for Neuroscience, Leioa Spain; 3. Research Unit in Experimental Medicine, national University of Mexico, Mexico; 4. Translational Neuropsychiatry Unit, Aarhus University, Aarhus, Denmark; 5. Pharmacology Department, Sao Paulo University, Riberaio Preto, Brasil; 6. Biobizkaia Health Research Institute, Baracaldo, Spain; 7 Biofisika Institute, Leioa, Spain ; 8.. Ikerbasque, Basque Foundation for Science

The prefrontal cortex (PFC) integrates emotional and cognitive functions. CB1 receptors are widely expressed in this region, principally on GABAergic interneurons, and participate in the Excitation-Inhibition (E-I) balance. E-I alterations are described in psychiatric disorders, including depression, anxiety, and schizophrenia, where a reduction of CB1 has also been described. Both the endocannabinoid system and mood disorders display important sex differences.

In this work, we aim to elucidate the role of CB1 receptors in prefrontal GABAergic interneurons in the development of behavioural disorders in a sex-dependent way. For this objective, we deleted CB1 from 1) all PFC neurons, 2) the GABAergic population and 3) in the Parvalbumin-positive (PV) subpopulation of GABAergic neurons of adult CB1-flox male and female mice. PV neurons were also modulated using an optogenetic approach in a cohort of animals. One month later, mice were subjected to several behavioral tests to evaluate their emotional and cognitive performance. The GABAergic neuronal activity was studied via calcium imaging by fiber photometry.

GABAergic CB1 deletion in the PFC altered the innate emotional behavior in both sexes but was much more pronounced in females, with no impact on memory. This effect correlated with lower activation of GABAergic neurons. In contrast, CB1 deletion restricted to PV GABAergic neurons produced opposite emotional impairments. Fear conditioning test revealed an impairment in learned-aversive responses in males and females. Immunofluorescence studies showed that global PFC CB1 deletion induced GABAergic-parvalbumin interneurons modulation with a sex-dimorphic pattern. Interestingly, the optogenetic modulation of these interneurons produced effects resembling those observed following CB1 deletion in PFC GABAergic neurons.

To summarise, PFC-GABAergic CB1 deletion induces an anxiogenic state and an exacerbated response to conditioned cues in a sex-dependent manner, potentially mediated by parvalbumin neuron activity. Thus, maladaptive prefrontal CB1 signalling could participate in the development of behavioral disorders.

10. Neuronal dynamics underlying depressive-like states.

Molina, **Patricia**[1], Osypenko, Denys[1], Garnica, Josep[2,3], Amossé, Quentin[1], Mondoloni, Sarah[1], Congiu, Mauro[1], Kathe, Claudia[1], Mameli, Manuel[1,4]

1 The Department of Fundamental Neuroscience, University of Lausanne, Lausanne, Switzerland; 2 Department of Oncology, Ludwig Institute for Cancer Research, Lausanne Branch, CHUV and University of Lausanne, Lausanne, Switzerland. AGORA Cancer Research Center, Lausanne, Switzerland; 3 Swiss Institute of Bioinformatics, Lausanne, Switzerland; 4 Inserm, UMR-S 839, Paris, France.

Chronic stress induces lasting neurobiological adaptations that can progressively lead to behaviors reminiscent of depressive states. However, the temporal dynamics of cellular and behavioral adaptations remain poorly understood. Altered neuronal activity in the lateral habenula (LHb) – a neural substrate encoding aversion and negative affect – has been implicated in stress-related behavioral effects and depression. Yet, the timing and trajectory of stress-induced LHb functional changes remain unresolved. Here, we mapped the temporal emergence of depression-related behaviors and their LHb neuronal correlates in mice undergoing chronic corticosterone (CORT) administration, a model mimicking prolonged stress hormone elevation.

Longitudinal two-photon calcium imaging to track the encoding properties of individual LHb neurons uncovered progressively increased responses to airpuff over CORT treatment. Analysis of ~500 LHb neurons identified clusters with stable excitatory or inhibitory responses, as well as dynamic clusters that gradually increased their response and exhibited spatial segregation within LHb. In parallel, behavioral phenotyping during CORT exposure revealed a gradual shift towards maladaptive states, characterized by reduced active coping and enhanced apathy-like behaviors. The emergence of these behavioral phenotypes paralleled the reorganization of LHb ensembles, suggesting coordinated circuit and behavioral adaptations. Remarkably, early intervention with ketamine reversed CORT-induced LHb remodeling, while chemogenetic inhibition of LHb neuronal hyperactivity within this critical window also normalized depression-related behaviors.

Thus, we identified a temporally defined and topographically organized remodeling of LHb neuronal ensembles during chronic stress that matched the onset of depressive behaviors and was reversed by timely antidepressant strategies. These findings could have implications for temporally tailoring therapeutic interventions.

11. Complex spike - simple spike dynamics predict behavioural encoding.

Bina L., Romano V., Bosman L.W.J., De Zeeuw C.I.
Erasmus Medical Center, Rotterdam, the Netherlands.

Purkinje cells in the cerebellum fire two types of action potentials: complex spikes (CSs) at low frequency and simple spikes (SSs) at higher frequencies. Both occur not only in response to stimuli or during movements, but also spontaneously at rest. When CSs are elicited, SSs pause for a few tens of milliseconds and then change transiently before returning to baseline. The post-pause dynamics of CS-SS interactions vary among cells, forming a continuum from strong suppression to pronounced facilitation of firing, yet it is unclear how they come about and to what extent they can be correlated with behaviour. Here, we show that the post-pause dynamics correlate with SS firing regularity during spontaneous firing, whereas duration of the pause itself correlates with both CS and SS firing rates. When we compared post-pause dynamics during different behavioural conditions, including reflexive whisker movements, treadmill walking, as well as uncued and cue-driven licking, they turned out to consistently correlate with SS modulation, but not with CS modulation. This relationship was stronger than that between pause duration and SS modulation. Moreover, the direction of the post-pause dynamics correlated with the kinematics of the movements in that the associated increases or decreases of SS modulation differentially encode the direction of the movements. The post-pause dynamics appeared to be mainly driven by intrinsic mechanisms of Purkinje cells in that their characteristics appeared to be consistent during spontaneous and stimulus-induced behaviour and that they depend on the expression of PP2B and GluR3, known to contribute to SS modulation up on parallel fiber input. These findings establish a Purkinje cell specific link between their CS-SS interactions and behavioural encoding, uncovering a new rule of engagement. Our results suggest that electrophysiological characterization of Purkinje cell firing properties at rest can be used to partially predict their cerebellar output during behaviour, revealing a previously unrecognized level of structure-function heterogeneity directly tied to cerebellar control of behaviour.

12. Inhibitory neuronal control of contextual fear memory persistence and precision.

Pablo Méndez, Nuria Cano-Adamuz, Rut de la Vega Ruiz, Alicia Caro Martín, César Redondo Alañón, Alicia Hernández-Vivanco,
1. Cajal Neuroscience Center, Madrid, Spain

In our everyday life, experiences are encoded in the brain in a manner that dynamically shapes our ability to recall them. Some events become rapidly unavailable for retrieval shortly after they occur, whereas others remain persistent and can be recalled over extended periods of time. At the same time, memory specificity and accuracy are adjusted by salience, attention or previous experiences and evolve throughout the time we retain the ability to recall them. Elucidating the neural mechanisms that regulate memory persistence and precision would enhance our ability to develop strategies to treat brain disorders characterized by impaired long-term memory formation or diminished memory specificity.

The hippocampus, a brain region essential for learning and memory, counts with a collection of inhibitory neuronal types (INs). INs use the neurotransmitter GABA to dictate the activity of other neurons and provide an extremely precise code that is used to process and store information. Here, focusing on genetically and anatomically defined hippocampal INs and using neuronal activity recordings, chemogenetics and unsupervised analysis of mouse behaviour during contextual fear conditioning, we studied the inhibitory neuronal mechanisms controlling memory persistence and precision. We show that a particular subtype of hippocampal IN, the somatostatin(SST)-expressing IN in the dentate gyrus (DG), plays different roles according to the phase of associative fear learning and processing. During memory formation, DG SST INs bidirectionally control the size of active neuronal ensembles and the temporal persistence of associative memories. In contrast, the activity of DG SST INs during memory recall limits associative fear memory extinction and gate the precision of the updated contextual fear memory. Overall, our findings uncover a novel circuit mechanism to adjust memory persistence and precision and offer timely insights into potential targets for therapeutic intervention in Alzheimer's disease and post-traumatic stress disorder.

13. Age dependent processing of spatial conflicts.

Liu Chen[1,2], Nuttin Bram[1,2], Hanson Laura[1,2], Hoy Jennifer[3] and Farrow Karl [1,2]
VIB – KU Leuven Centre for Neuroscience, Leuven, Belgium 1;
Department of Biology, KU Leuven, Leuven, Belgium 2;
Department of Biology, University of Nevada, Reno, USA 3;

Natural environments present animals with multiple competing visual cues. Selecting meaningful cues from distractors is a process shaped during animal maturation. This selection process is orchestrated by the superior colliculus (SC), a hub for sensorimotor integration, transforming visual information into actions. Within its topographic map, the superior colliculus mediates competitive interaction across visual space. However, it is unclear how its functional circuitry resolves conflicting ethological demands that require opposing motor action, such as freezing to an overhead threat versus approaching a foraging target. Furthermore, how this processing capacity matures from juveniles to adults remains elusive. Here we report that the spatial competition in the mouse superior colliculus acts as a filter that changes with age. Behaviorally, active engagement with the target attenuates the defensive response.Reciprocally, an overhead 'threat' suppresses approach toward a foraging target. This suppression of approach is stronger in juveniles than in adults. Using neuropixels recordings, we found that this behavioral inhibition correlates with a pervasive, age-dependent suppression of neural activity. In juveniles, the superior collicular neurons show stronger reduction in firing rates when conflicting cues are presented. In contrast, adult neurons maintain robust responses in the presence of conflict.

These findings suggest that the juvenile superior colliculus initially process spatial competition through broad, suppressive interactions, which are developmentally pruned to allow for less-interfering selection observed in adulthood. Collectively, this work reveals that maturation shapes neural resolution of spatial competition, explaining how sensorimotor circuits adapt to changing ethological demands of life.

14. Impact of age-related perturbations on a bump attractor model of working memory.

Antoniadou, **Alexandra** [1,2], Ibañez, Sara [1], Wimmer, Klaus [1,2]
1.Computational Neuroscience Group, Centre de Recerca Matemàtica, Barcelona, Spain; 2.Dept. of Mathematics, Universitat Autònoma de Barcelona, Barcelona, Spain

Normal aging in humans and non-human primates is associated with progressive cognitive decline in many cognitive tasks and particularly in working memory. Working memory is governed by the dorsolateral prefrontal cortex (dlPFC), a brain region that is undergoing pronounced alterations during normal aging, including myelin loss, synapse loss, and neuronal hyperexcitability. Despite a wealth of experimental data, a coherent theoretical framework of how these age-related neuronal changes interact and alter network dynamics is currently lacking. Here, we investigated how both the individual and shared contributions of aging factors can lead to working memory decline in bump attractor networks.

We propose a bump attractor network that models the dynamics of the dlPFC neural representations underlying spatial working memory, incorporating two key aging factors: myelination deficits and neuronal hyperexcitability. The model consists of excitatory and inhibitory populations of leaky integrate-and-fire neurons with sparse connectivity, that also incorporate short-term synaptic depression and facilitation (Hansel and Mato, 2013). Myelin loss was modeled as an increase in the action potential failure rate (Ibañez et al., 2023), and changes in excitability were introduced by modifications to the f-I curve of leaky integrate-and-fire neurons, fitted to empirical data (Ibañez et al., 2020).

Our models predict that biologically plausible levels of myelin loss and hyperexcitability account for substantial age-related working memory impairment via distinct mechanisms. Hyperexcitability causes a spread of neuronal activity and increased correlations within the network, leading to greater diffusion of the activity bump and less precise memory representations. In contrast, myelin loss primarily affects the amplitude, but not the width, of the activity bump, reducing firing rates and stability over time and thereby impairing memory duration. Furthermore, introducing a distractor at varying spatial locations and times revealed distinct effects on memory drift across network perturbations. In the hyperexcitable network, distractors that are spatially closer to the original stimulus and presented closer in time have a greater influence on drift. In contrast, in the demyelination-perturbed network, more distant distractors, both spatially and temporally, have a greater impact.

These findings highlight the different impacts of age-related changes on working memory circuit functionality, providing insights into the mechanisms of cognitive decline along with potential pathways for prevention and treatment.

15. A frontal-collicular circuit for routing visual distractions into goal-dependent behavior.

Song, **Kuisong**[1], Usseglio, Giovanni[1], Metaxas, Dimitrios[2], Cui, Peng[3], Morou, Sofia[1], Damoulas, Theo[4], Lazaridis, Iakovos[5], Femenia, Teresa[1], Kardamakis, Antonios Andreas[1]

1. Instituto de neurociencias, Alicante, Spain; 2. Medical University of Vienna, Vienna, Austria; 3. Karolinska Institutet, Stockholm, Sweden; 4. University of Warwick, Coventry, UK; 5. Massachusetts Institute of Technology, Cambridge, USA.

Can we predict when a visual distractor will capture our attention? Visual distractors can trigger overt responses or remain unattended. The circuitry underlying this action selection remains poorly understood due to the predominant use of paradigms restricted to head-restrained animals in multisensory tasks, while relevant visual behaviors in mice are centered around fear or escape responses. Here, we demonstrate a novel paradigm using self-initiated visual search to trigger animal navigation, enabling the randomized mid-flight presentation of salient visual stimuli to assess distractibility within the context of goal-dependent behavior. Computer vision methods were applied on imaging data to capture behavioral state transitions, which ranged from attentive distractor engagement to trajectory perturbations or stimulus neglect. To quantitatively characterize these behavioral states, we derived multiple kinematic metrics from the animals' movement patterns. This analytical framework enabled implementation of linear discriminant models to classify visual distraction intensity based on motion signatures during distraction phase, and to predict distraction level from preceding search-related behavioral patterns. During this attention-distractor paradigm, we manipulated and recorded from glutamatergic and GABAergic neurons in the intermediate layer of the lateral superior colliculus (l.SCi) to study whether this conserved circuitry is capable of tuning visual distractors in and out during behavior. In vivo cell-type-specific calcium recordings -via fiber photometry- revealed their involvement in this behavior, while optogenetic and chemogenetic manipulations enhanced or reduced their visual distraction routing. We also characterized modulation from upstream circuits, such as motor cortex to l.SCi projections, using the above methods. We propose that l.SCi glutamatergic and GABAergic neurons route visual distraction in an excitatory or inhibitory manner, subject to upstream-derived modulation during task-dependent behavior.

16. Balancing focus and distraction: the role of the superior colliculus in visual attention and distraction resilience.

Usseglio, **Giovanni** [1], Feugas, Pierre [1], Le Merre, Pierre [2], Song, Kuisong [1], Kardamakis, Andreas A [1].

1.Instituto de neurociencias, Alicante, Spain. 2. Centre de Recherche en Neurosciences de Lyon, CNRS UMR 5292, INSERM U1028, University Claude Bernard Lyon 1, Bron, France

Visual distraction routing is essential for the control of visuospatial attention and relies on coordinated interactions between cortical and subcortical structures, with the evolutionarily conserved midbrain superior colliculus (SC) occupying a central role. Previous work in the lab demonstrated, in freely moving mice, that responses to visual distractors can be predicted using a self-initiated visual search paradigm. Within this framework, randomized distractor presentation during ongoing movement uncovered distinct distraction-related behavioral states, quantified through kinematic metrics and classified using linear discriminant models. Cell-type-specific calcium recordings, together with optogenetic and chemogenetic manipulations, revealed that glutamatergic and GABAergic neurons in the intermediate lateral superior colliculus (l.SCi) route visual distraction. This routing is further shaped by upstream inputs, including projections from motor cortex, enabling task-dependent modulation of distractor engagement or suppression. Here, to obtain a mechanistic, circuit-level understanding of these processes, we developed a head-fixed visuospatial attention–distractor task to dissect how cortical regions modulate l.SCi activity during distraction routing. Using high-density, large-scale recordings with (i.e. Neuropixels), this study aims to provide an integrated view of the long-range cortico–subcortical circuits governing visual distraction.

17. Synaptic integration of visual distraction circuits in the midbrain.

Sofia Morou, Pierre Feugas, Giovanni Usseglio, Kuisong Song, Antonios Andreas Kardamakis
Instituto de Neurociencias, Universidad Miguel Hernandez, Alicante, Spain

Since early vertebrate evolution, neural circuitry embedded within the midbrain superior colliculus (SC) has evolved to support visual-based orienting behavior and is involved in salience detection and attention-distractor processes. The lateral division of the intermediate SC (l.SCi) contains both excitatory (E: vGluT2+) and inhibitory (I: vGAT+) neurons, serving as a brain-wide integration hub that receives visual, frontocortical, and nigral input. However, how these inputs interact to shape salience processing remains unclear. Here, we examine the integration modes and selective activation patterns of visual inputs arising from the retina and higher visual areas (HVAs), as well as frontal inputs (M1/M2), onto E and I neurons in the l.SCi using opto-electrophysiology. Using optogenetic stimulation combined with whole-cell patch-clamp recordings ex vivo, we assess whether convergent inputs cooperate through linear or nonlinear summation or compete via local inhibition. Additionally, using high-density Neuropixels recordings in the l.SCi, we investigate whether these inputs cooperate or compete, using patterned optogenetics of the two input areas. Our results indicate pathway-specific integration rules within the l.SCi. Inputs from HVAs preferentially activate excitatory neurons while bypassing local GABAergic populations. In contrast, motor cortical inputs robustly recruit both excitatory and inhibitory neurons, suggesting stronger engagement of local inhibitory circuitry. These findings reveal distinct modes by which cortical sensory and motor signals influence midbrain circuits and suggest that cooperation and competition between phylogenetically older SC circuits and neocortical inputs may play a key role in the computation of visual salience.

18. Investigating the brain substrates underlying higher-order social learning.

Irene P. Ayuso-Jimeno, Emanuela Iannella and Arnau Busquets-Garcia
Hospital del Mar Research Institute Barcelona, Spain

Adapting behavior based on context and past experiences is crucial for social animals. Social cues are key in this process as they help animals to learn about their environment and to interact with other conspecifics. Animals learn to indirectly associate low-salience sensory cues (conditioned stimuli, CS), such as sounds or lights, with salient stimuli (unconditioned stimuli, US) through higher-order conditioning processes, also known as mediated learning (ML). Recent studies have implicated the hippocampus (HPC) and the basolateral amygdala (BLA), among other brain structures, in ML. However, how these areas and other brain regions linked to social behavior, like the orbitofrontal cortex (OFC), contribute to socially induced ML and which specific cell types are involved remains fully unknown. Moreover, to our knowledge, no behavioral protocols for studying socially-induced ML are currently available. This project aims to systematically investigate the neural mechanisms underlying higher-order conditioning induced by social cues, focusing on a network of brain areas including OFC, BLA and HPC. To do so, we developed a new paradigm to test higher order conditioning induced by social defeat in male mice, where a low salience light and a tone (CS) are associated in a preconditioning phase, and then the light is devaluated by its pairing with a social defeat experience (US). After conditioning, direct and mediated learning are tested by presenting the light or the tone in a different context. By automatically characterizing behavior with unsupervised tools (DeepLabCut, Keypoint MoSeq), we have shown that mice learn to associate both the light (direct learning) and the tone (mediated learning) to the social defeat experience, suggesting that such an experience can trigger complex associations between context and past experience. Moreover, we have characterized the activity of excitatory neurons in BLA and OFC during all phases of this behavioral paradigm by performing fiber photometry experiments. Both BLA and OFC's activity raises significantly upon presentation of the light and the tone after conditioning with a social defeat experience, confirming our behavioral data. Future experiments will focus on the establishment of this protocol in female mice, the recording of a wider array of socially-relevant brain areas and cell types and the manipulation of such cell types in these regions to determine their roles in the formation and expression of direct and mediated learning. Overall, our work will provide new tools for studying higher-order conditioning in social contexts and deepen our understanding of how social experiences shape behavior.

19. Preferential vulnerability of cortical GABAergic interneurons to Nalcn deficiency.

Candela Barettino[1,2], Álvaro Ballesteros[1, 2], Yillcer Molina[1], Jorge Maldonado[1], Mario Soriano[2], Leszek Lisowski[3,4], Antonio Gil-Nagel Rein[5,6], Stephan Pless[7], Arnaud Monteil[8], Ramón Reig[1], Isabel del Pino[1, 2]

1 Institute of Neuroscience (IN), CSIC-UMH, 03550 Sant Joan d'Alacant, 03550 Alicante, Spain.

2 Centro de Investigación Príncipe Felipe, 46012 Valencia, Spain

3 Translational Vectorology Research Unit, Children's Medical Research Institute, Faculty of Medicine and Health, The University of Sydney, Westmead, New South Wales, Australia.

4 Laboratory of Molecular Oncology and Innovative Therapies, Military Institute of Medicine - National Research Institute, Warsaw, Poland.

5 Department of Neurology, Hospital Ruber Internacional, Madrid, Spain

6 Fundación Iniciativa para las Neurociencias (Fundación INCE), Madrid, Spain.

7 Department of drug design and Pharmacology, University of copenhagen, Copenhagen 2100, denmark.

8 IGF, Univ. Montpellier, CNRS, Inserm, F-34094 Montpellier, France.

Neuronal resting membrane potential (RMP) reflect the balance of leak conductances and varies systematically across cortical cell types. Many GABAergic interneurons exhibit more depolarized RMPs than neighbouring excitatory neurons, positioning them closer to spike threshold and enabling rapid inhibitory recruitment that shapes network dynamics. Yet, the molecular mechanisms underlying these systematic differences in intrinsic excitability remain incompletely defined. The sodium leak channels non-selective (NALCN) mediates a major fraction of basal sodium conductance and cause neurodevelopmental syndromes characterized by developmental delay and cognitive impairment. Here we define NALCN contribution to cortical circuit developmental and function with cell-type precision. Using Nalcn-GFP reporter line, we map Nalcn expression across cortical types revealing enrichment in GABAergic hippocampal interneurons relative to hippocampal pyramidal neurons. Using conditional mouse models, we delete Nalcn selectively in cortical glutamatergic lineages or forebrain GABAergic cells from early embryogenesis. Electrophysiological analysis show that developmental loss of Nalcn preferentially reduces intrinsic excitability across GABAergic interneuron subtypes while sparing pyramidal neurons. To understand the impact of a depolarized GABAergic RMP on brain function, we assess behavioral performance that revealed persistens deficits in contextual adaptation and spatial short-term memory. These findings position NALCN as a cell-type specific determinant of inhibitory excitability and cortical circuit function.

20. All-optical in vivo mapping of hypothalamic microcircuit plasticity following aversive social experience.

Mussetto Valeria [1], Ferrarese Leiron [1], Reverte Ingrid [2], Torchia Sofia [1], Girelli Leonardo [2], Gross Cornelius [1]

1 European Molecular Biology Laboratory EMBL Rome, Monterotondo, Italy

2 Sapienza University, Rome, Italy

The ability to learn and adapt in response to diverse social experiences is fundamental for animal survival. While key defensive actions against social threats such as flight or fight are innate, previous social experience can guide the selection of the appropriate response to minimize future harm, such as the development of social avoidance following social defeat. The adaptation to social defeat in animals may have clinical relevance, as extreme forms of maladaptive coping strategies in response to social adversity can contribute to the development of psychiatric disorders such as major depression and social anxiety. The ventromedial hypothalamus is a key brain structure in the innate defensive system, and activity in its ventrolateral part (VMHvl) can mediate both social aggression and escape, yet how previous experience influences hypothalamic social threat processing is not understood. Using a combination of in vivo and in vitro recording approaches, we investigated experience-dependent synaptic and neuronal plasticity in the VMHvl. Whole-cell patch-clamp recordings revealed evidence for a disruption of excitatory/inhibitory balance within the local VMHvl microcircuit. In addition, we optimized simultaneous calcium imaging and optogenetic manipulation in freely moving mice to minimize optical crosstalk, enabling concurrent recording and stimulation of local glutamatergic and GABAergic populations. This approach allowed us to longitudinally monitor changes in excitatory and inhibitory microcircuit connectivity over days, providing novel insights into the synaptic and circuit basis of hypothalamic adaptations to social defeat. Unravelling these physiological adaptations is a fundamental step towards elucidating how these adaptations deviate in pathological conditions, contributing to the development of psychiatric disorders.

21. Strategy-dependent organization of memory recall under interference.

Peixoto-Moledo ,Paula (Afiliation [1] and [2]); Mur, Angel (Afiliation [1]); Ahmadian ,Yashar (Afiliation [3])*; Jercog Pablo E.(Afiliation [1])* (*=equal contr.)
1: Centro de Neurociencias Cajal (CSIC), Madrid, Spain
2: Universitat de Barcelona, Barcelona, Spain
3: Department of Engineering, University of Cambridge, Cambridge, United Kingdom.

Memory-guided behavior requires animals to use past experience to make decisions that are more informed than the simple mapping of sensory-input into motor-output. In natural settings, recall often unfolds under conditions where multiple relevant memories compete, leading to interference and behavioral variability. Interference is typically interpreted as a loss or degradation of memory, yet it remains unclear whether it instead reflects structured internal processes governing how different memories are selected and therefore expressed, and how these processes are manifested at the level of neural population activity. Understanding how recall is organized when multiple memory traces coexist is thus central to linking memory, behavior, and internal state dynamics.

We addressed this question using the 8-ports maze (Morales et al., 2020), a rodent spatial task specifically suited to probe memory recall under interference, where multiple previously rewarded locations compete to guide behavior within the same session.

To move beyond average performance and characterize the internal organization of recall, we used a GLM-HMM to identify four latent recall strategies: memory-base-goal-directed (2h), outdated-memory (24h), memory-avoidance, and lapsus. These strategies dynamically fluctuated within and across sessions and explained trial-by-trial behavioral variability. We found that all inferred recall strategies were expressed during task performance, indicating that animals flexibly engaged multiple strategies to solve the task. But notably, interference only emerged through memory-guided strategies, indicating that recall failures reflect structured state selection rather than a global weakening of memory.

We have controlled our result interpretation by perturbing long-term memory through anti-NMDAR antibody infusion. We have observed a reorganized use of recall strategies and rigidity in strategy selection, with increased dominance of short-term recall and reduced expression of alternative strategies. This links synaptic dysfunction to changes in the large-scale organization of memory-guided behavior, rather than to a simple loss of stored information.

Building on this framework, ongoing calcium imaging analyses are demonstrating the presence of strategies related activity patterns in neural population dynamics. Together, this work reframes memory recall as a dynamic, state-dependent process, in which interference and variability arise from flexible strategy selection rather than memory decay and could be the first demonstration of strategy neuronal encoding in mouse hippocampus.

22. Beyond perception: assessing cortico-thalamic feedback dysfunction in developmental dyslexia.

Pérez-Montoyo, **Elena**; Andrés-Bayón, Belén; López-Bendito, Guillermina
Instituto de Neurociencias (IN, UMH-CSIC), San Juan de Alicante, Spain

Developmental dyslexia is a neurodevelopmental disorder primarily characterized by persistent reading difficulties and traditionally associated with alterations in language-related brain regions. However, increasing evidence indicates that dyslexia is also associated with dysfunctions in early sensory pathways, particularly affecting temporal processing in the visual and auditory domains. These findings challenge purely language-based accounts of the disorder.

According to predictive coding theories, perception emerges from the interaction between bottom-up sensory inputs and top-down predictions. Within this framework, cortico-thalamic feedback pathways play a critical role in shaping sensory representations by signaling mismatches between expected and actual sensory input. Previous human neuroimaging and behavioral studies have reported reduced connectivity within visual and auditory circuits, as well as impaired sensitivity to temporal changes in individuals with dyslexia compared to neurotypical readers. These observations indirectly point to alterations in predictive feedback mechanisms; however, the underlying causal and circuit-level substrates remain poorly understood.

Here, we address this gap using a mouse model that mimics key neurodevelopmental alterations associated with dyslexia, specifically impaired cortico-thalamic connectivity. As a first step, we assessed basic visual motion perception to determine whether feedforward sensory processing is preserved in this model. Our preliminary behavioral results indicate that basic visual motion perception is intact, supporting preserved feedforward visual processing.

We next investigate whether dyslexia-related alterations affect the ability to form and update predictions about dynamic visual stimuli. To this end, we are currently developing an experimental paradigm in which mice are exposed to repeated presentations of visual motion stimuli to establish stable expectations, followed by subtle or salient changes that violate these predictions. This ongoing work aims to determine whether dyslexia-related alterations selectively impact feedback processing rather than basic sensory encoding.

Together, this approach seeks to provide mechanistic insight into how disrupted cortico-thalamic communication may contribute to sensory processing alterations in developmental dyslexia, highlighting predictive processing as a unifying framework for understanding neurodevelopmental disorders.

23. Frontal Cortex Dynamics Reveal Temporal Misalignment Between Sensory Evidence and Choice.

Serrano-Porcar, **Balma** IDIBAPS; Rodríguez, Javier IDIBAPS; Herry, Cyril INSERM; Compte, Albert IDIBAPS; dela Rocha, Jaime IDIBAPS
IDIBAPS, Barcelona, Spain
INSERM, Bordeaux, France

In perceptual decision-making, sensory information is transformed into actions. Previous studies identified neural ensembles in the mouse frontal cortex that maintain the upcoming choice during the mnemonic delay (Guo et al. 2014). These findings are captured in a model where a dynamic attractor landscape emerges at stimulus onset and stabilizes the choice representation until the response (Inagaki et al. 2019). However, such models assume that stimulus presentation and decision timing are naturally aligned, an assumption that may fail when the timing between stimulus and response is unpredictable. To explore this hypothesis, we developed a three-choice visuospatial delayed response task for freely moving mice with variable stimulus and delay durations. Choice accuracy increased with stimulus duration and decreased with delay length, suggesting both perceptual and forgetting errors. Surprisingly, mice still responded in catch trials without stimuli, indicating an urgency signal that drives choices independently of sensory input, with the stimulus biasing—but not initiating—the decision. Motivated by this observation, we extended the two-choice attractor network model of Inagaki et al. 2019 to a three-choice circuit with three excitatory populations coupled through global inhibition. The network received two inputs: a non-selective urgency ramp and a transient sensory signal. In data-fitted models, task performance largely depended on the temporal alignment between the stimulus and urgency buildup. This reveals previously unconsidered errors occurring when the urgency peaks after stimulus offset (late-choice errors), leading to delayed choice encoding. To probe this prediction, we recorded population activity in the secondary motor cortex (MOs) and found that choice encoding at the population level emerged later in error trials. Together, our findings reinforce the idea that decision timing does not always align with the stimulus, especially under unpredictable conditions. Instead, other factors, such as the cognitive cost of maintaining a decision, may influence these late-choice dynamics.

24. Astrocyte-Dopamine Feedback Links Neuromodulation to Behavioral State.

Iakovos Lazaridis [1], Gun Ahn [1], Kojiro Hirokane [1], Sabrina Drammis [1], Wonchang Choi [2], Matsushima A [1], Hueske E [1], An M. Graybiel [1]

1, McGovern Institute for Brain Researchand Department of Brain and CognitiveSciences, Massachusetts Institute of Technology, Cambridge, MA, USA. 2, Department of Computer Science andEngineering. Sogang University, Seoul, Republic of Korea.

Behavioral flexibility is the ability to adjust actions as internal state and environmental demands change. It is essential for adaptive decision-making and is strongly shaped by dopamine. In the dorsal striatum, dopamine influences action selection and learning, but it remains unclear how dopaminergic signals are translated into shifts in behavioral state. Astrocytes are increasingly recognized as active participants in neuromodulatory processing, raising the possibility that dopamine shapes behavior not only by acting on neurons directly, but also by acting through astrocytes to influence the broader striatal environment.

Here we describe an astrocyte-dopamine feedback motif in dorsal striatum that links neuromodulation to behavioral state. During a self-initiated, reinforcement-guided decision-making task, astrocytic calcium activity showed little evidence of encoding specific choices or outcomes. Instead, large astrocytic calcium events were most prominent during task-unengaged periods and clustered around transitions back into sustained task performance, often preceding renewed trial initiation. Dopamine fluctuations were structured around the same transitions and typically rose earlier than astrocytic events, consistent with a sequence in which dopamine recruits astrocytes during state change. Aligning behavior to astrocytic events revealed a coordinated shift from task-unrelated to task-relevant behavioral motifs, and decoding analyses likewise indicated that astrocyte signals were more informative about behavioral state than about trial outcome.

Mechanistically, evoked dopamine release robustly activated striatal astrocytes through an astrocytic Drd2-dependent pathway, and direct activation of striatal astrocytes suppressed local dopamine levels without altering overt locomotion. Together, these findings support a model in which dopamine signals through astrocytic Drd2 to recruit a feedback program that regulates dopamine availability and resets the functional state of striatal networks. A plausible effector is astrocyte-derived adenosine, which could reduce dopamine release and shift the balance of striatal pathway excitability by dampening D1-favoring drive while enhancing D2-favoring influence. In this framework, astrocyte-dependent suppression of dopamine acts as a network reset signal that reconfigures D1/D2 competition and supports transitions between behavioral states, providing a glial mechanism for behavioral flexibility.

25. Stage-dependent cerebrocerebellar communication during sensorimotor processing.

Romano V.
Erasmus MC

Cerebral cortex and cerebellum are essential for sensorimotor control, but the dynamics of their interactions remain unclear. Here, we investigated which pathways prevail during preparation and execution of spontaneous whisker movements in mice. During preparation, neuronal activity of primary motor (M1) and somatosensory (S1) cortex precede that of cerebellar crus regions, with a lead that is consistent with relaying a copy of motor commands. After movement onset, the phase of the signal inverts, indicating a dominant vector signaling from cerebellum to cerebrum. At this stage, Purkinje cell activity correlates more with S1 than M1, generating a prediction of sensory consequences during motor actions. A computational cerebello-cortical model could replicate the changes in dynamics and directionality. Optogenetic manipulations of pons and thalamus confirm the modeled predictions on stage-dependent dynamics. Together our data point towards a swap in direction of information flow between cerebrum and cerebellum when motor preparation switches to execution.

26. Cortical Representation of Touch: Transfer of Perception from Artificial to Natural Stimulation.

Alexandre Tolboom, Noé Poncet (presenting author), Isabelle Férézou, Valérie Ego-Stengel
NeuroPSI, CNRS and Université Paris-Saclay

Stimulation of human brain can evoke perception of sensory stimuli, for example perception of phosphenes. In rodents, artificial stimulation of the primary somatosensory cortex (S1) can replace whisker touch during tactile discrimination tasks. Of particular interest, optogenetic methods now allow to apply precisely defined spatiotemporal patterns on the known topography of sensory cortices, thus mimicking natural spatiotemporal patterns evoked by peripheral stimuli.

In this project, we want to characterize how similar optogenetic and peripheral stimulation are, in terms of perception and integration into behavior, and in terms of the underlying neural activity.

To address these objectives, we took advantage of the well-known whisker system of the mouse. To manipulate neuronal activity in the barrel cortex (whisker S1), we induced the expression of channelrhodopsin in excitatory neurons. We observed that mice could learn to detect a 300-µm, 500-ms optogenetic spot projected on the barrel cortex, and could discriminate between two spots with centers 150 µm apart or more, even if overlapping.

Previously, we trained mice to discriminate the position of an optogenetic bar rotating on the barrel cortex (Lassagne et al., 2022). In this project, we show that mice can similarly learn to discriminate the position of a physical bar rotating across the whiskers.

We further interrogate the ability of mice to transfer such perceptual learning from artificial to natural stimulation conditions, in a context where tactile inputs are instrumental in guiding motor learning. We implemented a closed-loop brain-machine interface in which mice learned to control the position of a virtual cursor by modulating the activity of primary motor cortex neurons (similar to Goueytes et al., 2022). Mice were guided by an optogenetic feedback, which was a light bar projected on the barrel cortex. When switching to a peripheral bar feedback, mice managed to control the virtual cursor at the first session, demonstrating a rapid transfer across stimulation modalities.

Finally, to understand how this transfer was possible, we recorded the spatio-temporal dynamics of evoked activity in the barrel cortex of anesthetized mice using voltage-sensitive dye imaging. The rotation of a bar across the whiskers evoked a sequence of blobs, whose location moved on the barrels topography in synchrony with the bar.

Together, these results demonstrate that cortical optogenetic stimulation, when based on somatotopic maps, can reproduce certain components of activity caused by natural stimulation, inducing reliable tactile perception.

27. Norepinephrine and acetylcholine joint control of amygdala dynamics.

Alegre Cortés, Javier [1,2], Karalis, Nikolas, [1,2]
Paris Brain Institute, Paris, France; INSERM

Acetylcholine (ACh) and norepinephrine (NE) critically regulate internal states such as arousal. Although their effect on ionic currents is established, how NE-ACh interactions shape network dynamics and brain state transitions remain unclear. To answer this question, we leveraged experimental and computational methods to explain NE-ACh interactions in the amygdala, a key region for emotion and learning.

Combining NE and ACh monitoring with large-scale in vivo electrophysiology in awake mice, we characterize how the joint action of the two neuromodulators influenced neuronal activity. We clustered the neurons according to their latent dynamics and quantified the sensitivity of each neuronal subpopulation to independent and joint neuromodulation. Contrary to the general excitatory effects described ex vivo, NE and ACh release triggers heterogeneous responses across neurons in vivo. Interestingly, a specific cluster of ~30% of the neurons exhibits an additive increase in firing when both NE and ACh are released, indicating a synergistic action of the two neuromodulators.

To explain the combined mechanism, we created a mean-field model that incorporates the effects of NE and ACh on excitability and synaptic properties. Using bifurcation analysis, we identified that the combined action of ACh (facilitating presynaptic release) and NE (increasing neuronal gain) recapitulates electrophysiological signatures observed in vivo. Importantly, we could predict effects not explicitly implemented, including enhanced firing of inhibitory neurons and reduced activity in a subset of excitatory neurons.

In summary, we characterized how NE-ACh interactions shape amygdala activity during behavior and generated testable predictions on the mechanistic underlying their effect on neuronal dynamics.

28. The effect of systemic ketamine on working memory history dependencies.

Chatzimichail, Konstantinos [1 2], Tschiersch, Melanie [1 2], Zhu, Junda [3], Wang, Zhengyang [3], Mahrach, Alexandre [1], Constantinidis, Christos [3], Compte, Albert [1 4].
1. Institut d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS), Barcelona, Spain; 2. Universitat de Barcelona, Spain; 3. Vanderbilt University, Nashville, Tennessee, United States; 4. Institut d'Investigacions Biomèdiques de Barcelona, IIBB-CSIC, Barcelona, Spain

Working memory (WM) responses in primates are attracted to immediate prior stimuli (serial dependence) and to the mean of the long-term distribution of stimuli (long-term history dependence). It has been shown that WM attractive serial dependence is reduced in people with schizophrenia or autoimmune anti-NMDAR encephalitis, diseases linked to hypofunctional NMDARs[1,2], and in autism, while people with dyslexia show instead a reduction in long-term history dependence[3]. These distinct patterns across brain disorders underscore the relevance of understanding the mechanisms of WM history-dependent biases. Here, we study the mechanisms underlying WM serial and long-term history dependence in four macaque monkeys performing a biased visuospatial oculomotor delayed response task. In each session, stimuli followed a bimodal Gaussian distribution, with two diametrically opposed mode locations, which varied by session. In some sessions, monkeys were administered ketamine, an NMDAR antagonist, systemically. We used linear models to assess the serial and long-term history dependence of the monkeys' responses. Surprisingly, monkeys exhibited mostly repulsive serial and long-term history biases. Ketamine reduced repulsive serial dependence but increased repulsive long-term history bias, suggesting distinct mechanisms for the two processes. Moreover, to investigate candidate circuit mechanisms, we trained excitatory–inhibitory recurrent neural network models to perform the same task under identical stimulus statistics. The models incorporated biophysically motivated mechanisms, including adaptation, synaptic plasticity, and NMDA receptor–mediated currents. To mimic the effects of ketamine, we perturbed the trained networks by removing NMDA currents and reducing adaptation. Simulations of the behavioral task with these perturbed networks reproduced the differential effects of ketamine on serial and long-term history dependence, providing a mechanistic hypothesis for the distinct effects of ketamine on these two forms of working memory bias.

[1] Stein, H. et al. Nat. Commun. 11, 4250 (2020).
[2] Bansal, S. et al. Biol. Psychiatry Cogn. Neurosci. Neuroimaging (2023)
[3] Lieder, I. et al. Nat. Neurosci. 22, 256–264 (2019)

29. Dual encoding of slow and fast fluctuations in task-related vigor in rat dorso-medial striatum.

Hernández-Navarro, Lluís [1 2], Hermoso-Mendizabal, Ainhoa [1], Bektic, Lejla[1], de la Rocha, Jaime [1], Hyafil, Alexandre [2].

1. Centre de Recerca Matemàtica, Bellaterra, Spain;
2. IDIBAPS, Barcelona, Spain.

Interacting with the environment requires not only planning sequences of actions but also setting the duration of each step in the sequence, based on time, energy, and cognitive constraints. Here we studied in a perceptual decision-making task how rats set the time to initiate a choice response ("reaction time", RT), perform the response movement ("movement time", MT) and initiate a novel trial ("inter-trial interval", ITI). All three behavioral epochs co-varied strongly within each session and across sessions, reflecting fluctuations in motivation and fatigue. These co-fluctuations were captured by a single latent dimension that we term "slowly varying vigor", which was inferred using Gaussian Process Factor Analysis (GPFA). Strikingly, this motivational variable explained a much larger part of the variability of all three behavioral epochs (RT, MT, ITI) than cognitive variables such as sensory evidence, history-based expectations, or the previous outcome. Moreover, we found that the firing rate of a large proportion of individual neurons in the dorso-medial striatum (DMS) tracked the slow fluctuations of vigor across trials and sessions. Time-resolved regression analyses revealed a double dissociation in DMS activity: while the slowly varying component of vigor was encoded by tonic activity in the population, the variability of each behavior epoch above this slow component was encoded by phasic responses in DMS around the corresponding epoch. Our computational framework to dissociate slow and fast variations of vigor in decision-making tasks sheds a new light on the function of striatum in motivating behavior.

30. Locally balanced inhibition allows for robust learning of input-output associations in feedforward networks with Hebbian plasticity.

Cecchini, **Gloria**, Roxin, Alex
Centre de Recerca Matematica, Barcelona, Spain

In neural networks within the brain, the activity of a post-synaptic neuron is determined by the combined influence of many pre-synaptic neurons. This distributed processing enables mechanisms like Hebbian plasticity to associate sensory inputs with specific internal states, as seen in feedforward structures such as the CA1 region of the hippocampus. By modifying synaptic weights through Hebbian rules, sensory inputs can subsequently elicit outputs that consistently reflect their corresponding internal states. When input and output patterns are uncorrelated, this approach allows for the encoding of a large number of distinct associations, enabling efficient memory storage.

Our study demonstrates a critical limitation when output patterns become weakly correlated with input patterns through the intrinsic feedforward network's connectivity. In these cases, the Hebbian rule preferentially strengthens synaptic weights shared across patterns, leading to a "freezing" of the network's structure. This results in highly correlated output patterns over time, effectively reducing the network's capacity to store diverse associations and limiting its flexibility in learning.

To address this challenge, we find that including a mechanism of locally balanced inhibition, which has been shown to be a key feature of cortical circuits in-vivo, counteracts the undesired correlations between inputs and outputs. By dynamically regulating inhibitory input, locally balanced inhibition prevents the over-strengthening of shared weights, restoring the network's ability to maintain robust and flexible learning. This finding underscores the importance of inhibitory mechanisms in enabling efficient and adaptive information processing in neural circuits, offering insights into how biological networks maintain their remarkable capacity for associative learning.

31. Cell-type specific neuromodulatory dynamics underlying flexible behavior.

Serra, **Irene**[1], Karalis, Nikolas[1,2]
1 Paris Brain Institute, Paris, France; 2 INSERM

At any moment, distinct combinations of neuromodulators are released throughout the brain, critically shaping circuit dynamics and behavior. These signals act in a cell-type–specific manner, differentially modulating neurons and astrocytes, through distinct signaling mechanisms that operate across diverse spatial and temporal scales. How such parallel neuromodulatory pathways are coordinated in vivo, and how their cell-type–specific effects are integrated to regulate cognitive behavior, remains largely unknown.

Here, we use multiplexed fiber photometry to simultaneously monitor neuromodulator release and cell-type–specific population activity in the mouse prefrontal cortex (PFC), a central hub for flexible and adaptive behavior. We recorded astrocytic calcium activity alongside local neuromodulator dynamics using distinct G-protein-coupled Receptor Activation-Based (GRAB) sensors for the main neuromodulatory systems, including serotonin, norepinephrine, acetylcholine, and dopamine while freely behaving mice performed a cognitively-demanding automated rule-switching task.

Using this data, we have characterized the dynamics of prefrontal neuromodulation and its relation to astrocytic population activity at multiple timescales, ranging from event-locked responses to latent state modeling. We show that distinct neuromodulatory systems exhibit dissociable relationships with astrocytic activity across behaviorally relevant events, contingency changes, and transitions between behavioral states.

By integrating neuronal activity of prefrontal populations, these findings work towards delineating how major neuromodulatory inputs differentially relate to neuronal and astrocytic substrates to support adaptive behavior, offering a unified mapping of neuromodulator dynamics and cell-type–specific population activity during complex behavior.

32. Cooperative actions of interneuron families support the hippocampal spatial code.

Abad-Pérez, **Pablo** [1], Gallardo, Andrea [1], Picco , Marta [1], Garcia-Hernandez, Raquel [2], Brotons, Jorge [2], Martínez-Félix, Anel, [1], Machold, Robert Neuroscience Institutute, New York University, New York, USA, Rudy, Bernardo, [3], Buzsaki, Gyorgy, [3], Valero, Manuel [1]

1. Hospital del Mar Medical Research Institute, Barcelona, Spain; 2. Instituto Neurociencias UMH-CSIC, Alicante, Spain; 3. Neuroscience Institutute, New York University, New York, USA

Identifying the computational roles of different types of neurons is a prerequisite for understanding neural networks. Most of this cortical neural diversity is embodied in various types of GABAergic interneurons, grouped into four major families: Pvalb, Sst, Vip, and Id2 neurons. We have collected an extensive dataset of opto-tagged neurons from all four families, along with Camk2-expressing excitatory neurons, from the neocortex and hippocampus. The physiological features of the opto-tagged neurons were used to train a machine learning classifier, which was subsequently employed to accurately infer specific interneuron types in large-scale recording experiments. By combining targeted optogenetic manipulations with neuron type classification, we reconstructed the circuit's synaptic connectome. The simultaneous activity of interneurons predicted the animal's position to the same extent as excitatory neurons, with distinct contributions from different types. Using both functional connectivity analyses and targeted optogenetic manipulations, we demonstrate that interneuron types differentially control the magnitude of spiking within the place field and contribute uniquely to the different features of place fields of pyramidal neurons. Our findings assign a prominent role of interneurons to the flexible cognitive map.

33. Tuning the latency of tactile feedback in a mouse brain-machine interface for optimal learning.

Valérie Ego-Stengel, Alexandre Tolboom, Henri Lassagne, Daniel Shulz, Luc Estebanez - NeuroPSI, CNRS & Université Paris-Saclay, France

Brain-Machine Interfaces (BMIs) aim to improve patient autonomy. Beyond restoring movement, fine control of prosthetic devices requires restoring tactile sensory feedback. While BMIs with artificial somatosensory inputs have recently been used in patients, few studies have explored the spatio-temporal constraints of feedback integration.

This project examines how temporal latency between motor commands and sensory feedback affects control. We developed an ultra-fast bidirectional BMI using chronic recordings from whisker-related primary motor cortex (wM1) and 2D patterned optogenetic stimulation of whisker primary somatosensory cortex (wS1) in mice.

We designed a behavioral task where single wM1 neuron spikes controlled the rotation of a virtual bar. A photostimulation pattern on wS1 provided feedback about the prosthesis angle during a reaching task. Our incremental algorithm enabled fine control, with well-guided trajectories achieved using a 50-ms feedback latency. Altering this latency to 5 or 500 ms disrupted the animals' ability to move and stabilize the prosthesis, suggesting a critical time window for S1-M1 interaction.

We also explored the sensations evoked by optogenetic wS1 stimulation. After the mice mastered the BMI task with cortical stimulation, we replaced it with physical stimulation using a moving bar on the whisker array. This peripheral input targeted body regions corresponding to the previously stimulated cortical sites. The mice retained performance without relearning. Thus, S1 optostimulation appears to evoke perceptions similar to real tactile inputs, highlighting its biomimetic potential.

34. Unraveling inhibitory connections shaping functional connectivity in hippocampal CA1 in vivo using large-scale extracellular recordings and high-resolution optogenetics.

Picco Marta,[1] Gallardo Andrea,[1] , Martin-Fernandez Mario, [2] ,Mederos Sara,[1] ,Valero Manuel,[1]
1: Hospital del Mar Research Institute, Barcelona, Spain
2: Cajal Institute CSIC, Madrid, Spain

Hippocampal CA1 neuronal dynamics are fundamental for episodic memory formation and consolidation. Inhibition is expected to play a key role in organizing memory representations by regulating these dynamics. Inferring monosynaptic inhibitory connections in vivo from extracellular recordings remains challenging, as inhibition lacks direct signatures and is embedded within complex circuit architectures. To address this challenge, we present a method to infer inhibitory monosynaptic connections in hippocampal CA1 in vivo using high-resolution optogenetics. We reasoned that pyramidal cells become less excitable when synaptically inhibited by interneurons. This principle enables the inference of inhibitory connections from reductions in pyramidal cell responses to external stimulation that converges on the same neuron during interneuron activation. We first validated our approach using neural network simulations and whole-cell paired recordings in vitro. Then, we scaled up our method using large-scale recordings and high-resolution optogenetics in the hippocampus of freely-moving mice. We examined all possible interneuron-pyramidal pairs. By combining excitatory and inhibitory connections, we can generate an accurate functional connectivity map of the recorded hippocampal circuit. This enables quantitative analyses of the local connectome allowing comparison between functional and structural connectivity. Studying these relationships provides deeper insight into how synaptic connections are organized and how their patterns may change during learning. This work establishes a novel approach to reveal inhibitory connections in vivo, enabling a more complete mapping of hippocampal functional connectivity using large-scale recordings and high-resolution optogenetics. Future studies will extend this framework to simultaneously identify excitatory and inhibitory connections and investigate synaptic dynamics during learning.

35. A framework for adaptive temporal weighting across behavior and neural network models.

Ferro, **Demetrio** [1], Vivar, Citlalli [1], Levi, Aaron [2], Huk, Alexander [2], Wimmer, Klaus [1,3]
1: Centre de Recerca Matemàtica, Campus Bellaterra, Barcelona, Spain;
2: Departments of Ophthalmology and Psychiatry & Biobehavioral Sciences, Fuster Laboratory for Cognitive Neuroscience, UCLA, Los Angeles, United States.
3: Departament de Matemàtiques, Universitat Autònoma de Barcelona, Barcelona, Spain.

Perceptual decision-making relies on the accumulation of sensory evidence over time to form discriminations. Classical models link this process to distinct psychophysical effects, including primacy-related early weighting, uniform integration, and recency-related weighting arising from leaky integration. However, experiments show that humans and non-human primates can flexibly adapt temporal weighting strategies to stimulus statistics, a capacity that current models of cortical dynamics do not fully explain.

We found that macaque monkeys learn different temporal weighting strategies through exposure to a motion discrimination task with stimulus statistics (Levi et al., Journal of Neuroscience 2023). Their pre-stimulus vigilance co-varied with the temporal weighting profile, highlighting the role of internal state in evidence accumulation. Moreover, analyses of middle temporal (MT) cortex reveal that while average population firing rates remain stable across weighting strategies, stimulus-related and choice-related activity depends on stimulus statistics in non-trivial ways.

To explain these findings, we introduce a two-area firing rate model comprising interconnected sensory and decision circuits (Wimmer et al., Nature Communications 2015). A modulatory signal regulates the attractor dynamics of the decision circuit, initiating evidence integration and shaping decision timing. By varying manipulating this signal, the model reproduces early, flat, and late temporal weighting. Bidirectional connectivity dissociates choice probability (CP) into early stimulus-driven and late decision-related components, reproducing distinct CP time courses across conditions.

Finally, task-optimized recurrent neural networks (RNN) show that introducing contextual signals enables flexible temporal weighting, faster learning, and generalization, identifying contextual modulation as a unifying mechanism for adaptive temporal integration.

36. Synaptic Depotentiation and Network Dynamics as Physiological Correlates of Forgetting.

Moreno, Andrea [1,2,3,4];Mosqueira, Alejo [1,2,3,6];Nazari, Milad [1,2,3];Ansarifar, Sanaz [1,2,3];Faress, Islam [1,2,3];Mani, Leonardo [1,2,3];Kajendra, Nick [1,2,3];Chattarji, Sumantra [5];
Nykjaer, Anders [1,2,4];Nabavi, Sadegh [1,2,3]
1 Danish Institute of Translational Neuroscience (DANDRITE), Aarhus University, Aarhus, Denmark
2 Center for Proteins in Memory (PROMEMO), Aarhus University, Aarhus, Denmark
3 Department of Molecular Biology and Genetics, Aarhus University, Aarhus, Denmark
4 Department of Biomedicine, Aarhus University, Aarhus, Denmark
5 National Centre for Biological Sciences, Tata Institute for Fundamental Research, Bangalore, India
6 School of Life Sciences, University of Nottingham, Nottingham, United Kingdom

Memories are believed to persist through activity-dependent changes in synaptic strength, yet the synaptic and circuit mechanisms underlying memory weakening over time remain poorly understood. A key limitation has been the difficulty of repeatedly tracking memory strength alongside synapse-specific physiological readouts as memories naturally decay. Here, we address this challenge by combining refined aversive conditioning with optogenetic tagging and manipulation of thalamo–amygdala inputs in mice, enabling longitudinal behavioural, synaptic, and network-level analyses of forgetting. We establish a conditioning protocol that produces a robust fear memory detectable at 24 hours but that progressively decays over approximately one week. This decay is accompanied by a marked reduction in conditioned freezing and a parallel decrease in light-evoked field excitatory postsynaptic potentials (fEPSPs) at tagged thalamo–amygdala synapses. Critically, this recall failure is reversible: optogenetically induced long-term potentiation (LTP) restores both synaptic efficacy and behavioural expression, even when applied days after learning. In contrast, extinction induced by repeated unreinforced cue presentations leads to a similar behavioural suppression that cannot be rescued by LTP, indicating that decay and extinction rely on distinct mechanisms. Longitudinal recordings using chronically implanted opto-electrodes did not detect a sustained increase in synaptic strength at these inputs following learning, within the sensitivity and temporal resolution of our measurements, whereas forgetting was associated with synaptic depotentiation. Recovery of memory expression following LTP is accompanied by persistent increases in fEPSP amplitude and slope. Frequency-domain analyses further show that memory decay is associated with alterations in gamma-band activity during cue presentation, which are restored by synaptic potentiation but remain suppressed after extinction. Together, these results identify synaptic depotentiation and altered gamma dynamics as core physiological correlates of memory decay and demonstrate that forgetting reflects a weakening of synaptic connections, clearly distinguishing decay from extinction at behavioural, synaptic, and network levels.

37. Pupil size modulation drives retinal activity in mice and shapes human perception.

Santiago B. Rompani [1], Tjasa Lapanja [1], Pietro Micheli, Andrés González-Guerra [1], Oleksandr Radomskyi [1], Gioia De Franceschi [1], Josephine Jüttner [2], Petri Ala-Laurila [3], Georg Keller [4], Gabriel Peinado Allina [3], Hiroki Asari [1].

1. Epigenetics and Neurobiology Unit, EMBL Rome, European Molecular Biology Laboratory, Monterotondo, 00015, Italy;
2. Friedrich Miescher Institute for Biomedical Research, Basel, Switzerland;
3. Faculty of Biological and Environmental Sciences, Molecular and Integrative Biosciences Research Programme, University of Helsinki, Helsinki, Finland;
4. Faculty of Science, University of Basel, Basel, Switzerland

Retinal adaptation is assisted by the pupil, with pupil contraction and dilation thought to prevent global light changes from triggering neuronal activity in the retina. However, we find that pupillary constriction from increased light, the pupillary light reflex (PLR), can drive strong responses in retinal ganglion cells (RGCs) in vivo in mice. The PLR drives neural activity in all RGC types, and pupil-driven activity is relayed to the visual cortex. Furthermore, the consensual PLR allows one eye to respond to luminance changes presented to the other eye, leading to a binocular response and modulation during low-amplitude luminance changes. To test if pupil-induced activity is consciously perceived, we performed psychophysics on human volunteers, finding a perceptual dimming consistent with PLR-induced responses in mice. Our findings thus uncover that pupillary dynamics can directly induce visual activity that is consciously detectable, suggesting an active role for the pupil in encoding perceived ambient luminance.

38. Synaptic and Cellular Mechanisms Underlying Association of Events Separated in Time.

Nabavi, **Sadegh**[1,2,3]; Lima, Joao[1,2,3]; Mosqueira, Alejo[1,2,3]; Faress, Islam[1,2,3]; Barta, Anna[1,3]; Piriz, Joaquin[4,5]; Kvitsiani, Duda[1,3]

1.Danish Institute of Translational Neuroscience (DANDRITE), Aarhus University, Aarhus, Denmark.

2. Center for Proteins in Memory (PROMEMO), Aarhus University, Aarhus, Denmark.

3. Department of Molecular Biology and Genetics, Aarhus University, Aarhus, Denmark.

4. Achucarro Basque Center for Neuroscience, Leioa, Spain.

5. Ikerbasque - Basque Foundation for Science, Bilbao, Spain.

The brain can associate events that are separated in time. This feature appears to contradict dominant cellular models of learning, which require near coincidence of events for effective association. While persistent neuronal activity in the medial prefrontal cortex (mPFC) is hypothesized to bridge these temporal gaps, the underlying cellular and synaptic implementation remain unclear. To investigate this, we used trace fear conditioning, a form of associative learning in which the tone and shock are separated by many seconds. By tracking putative monosynaptic connections between the neurons in the mPFC from acquisition through recall, we identified three outstanding features of the underlying mechanism: the aversive stimulus (shock) induced an immediate and long-lasting increase in spontaneous activity in a subpopulation of neurons. This subpopulation of shock-responsive neurons with heightened excitability was more likely to strengthen monosynaptic connections with presynaptic tone-responsive neurons, and subsequently, to be activated by the tone during recall. Among tone-responsive presynaptic neurons, those that maintained elevated firing by the delivery of the shock contributed more to the formation of the association. Our data support the notion that the association of events separated in time can be implemented through a Hebbian form of plasticity.

39. Neural Mechanisms of Evidence Accumulation in Continuous and Categorical Perceptual Decision Making.

Crespo, **Pablo** [1], Wilson, Anna [1], Murphy, Peter [2], Wimmer, Klaus [1]
1. Centre de Recerca Matemàtica, Barcelona, Spain
2. Dept. of Psychology, Maynooth Univ, Dublin, Ireland

Perceptual decision making involves categorical choices, whereas continuous estimation tasks require ongoing computation of continuous variables. Despite shared reliance on temporal evidence accumulation, the neural differences between these processes remain poorly understood.

We investigated the temporal integration of sequential visual information using a circular spatial-mean task. Participants (N=48) performed continuous or categorical (binary) estimations of the mean location of a sequence of stimuli presented along a circular trajectory. EEG and pupillometry data were recorded during the experiment.

Subject estimations varied systematically, with performance declining as task difficulty increased. To quantify this, we developed a statistical framework that models evidence integration as the vector sum of stimulus orientations, incorporating systematic biases and stochastic noise. Fitting the model to single-trial data using Maximum-Likelihood estimation, we identified: (1) a temporal bias, with early stimuli being overweighted (primacy) in most participants; (2) an anisotropic spatial bias, favoring horizontal (left/right) over vertical stimulus locations; and (3) a systematic up-weighting of stimuli that deviated from the running average (a surprise-dependent bias). We demonstrate that this deviant sample up-weighting effect, in combination with the noise component, accurately predicts subjects' errors.

Brain activity (EEG) revealed that Centroparietal Positivity (CPP) signals, an evidence accumulation hallmark, are modulated by stimulus deviation from the running average, providing a neural correlate for the observed deviant up-weighting effect. Furthermore, we successfully decoded stimulus orientation sequences from alpha-band oscillations.

Ongoing work aims to dissociate the neural signatures of specific error sources to elucidate the neural basis of suboptimal choices across task types.

40. Parallel neural pathways for perceptual decision making in concurrent bilateral motion discrimination.

Tang, Haowen, Wimmer, Klaus, Hyafil, Alexandre.
Centre de Recerca Matemàtica, Bellaterra, Spain

What are the neural processes that allow us to monitor perceptually challenging sensory information across the visual field and act accordingly? Here we examine how the brain processes competing visual motion streams concurrently in the face of varying lateral stimulus strengths, and how it integrates evidence to form a decision. Participants simultaneously monitored two continuous displays of random dot kinematograms on both sides of the screen and were asked to report upward motion on either side by pressing the corresponding button. We find that the subjects' behaviour is captured by a race model that integrates sensory evidence towards each possible target in two parallel processes. EEG signatures are consistent with this concurrent integration of two motion streams along sequential processes: (i) The early target selection signal N2 tracks the coherence of contralateral motion, but does not discriminate between targets and non-targets; (ii) In contrast, a later parietal component responds stronger to motion evidence indicating a target than a non-target; (iii) Crucially, while Centro-Parietal Positivity (CPP) has been previously described as indexing integrated evidence towards decision formation, we find that these parietal components are segregated laterally, pointing towards a more spatially differentiated accumulation process. This study extends our understanding of functional roles and interactions of target selection mechanisms and evidence accumulation of multiple visual stimuli in perceptual decision making.

41. Astrocytes gate hippocampal sharp-wave ripples via cell-type-specific inhibitory circuits.

del Castillo-Berges, Ignacio, [1], Martinez-Felix, Anel, [1], Abad-Perez, Pablo, [1], Mederos, Sara, [1], Martin-Fernandez, Mario, [2], Valero, Manuel, [1]

Hospital del Mar Medical Research Institute, Barcelona Biomedical Research Park, Barcelona, Barcelona, Spain ; Centro de Neurociencias Cajal (CNC), Consejo Superior de Investigaciones Científicas (CSIC), Madrid, Spain

A growing body of evidence highlights the importance of distinct neuronal populations in hippocampal networks for learning and memory, and how their interactions shape circuit function. A paradigmatic example of such coordination is sharp-wave ripples (SPW-Rs), transient hippocampal network events arising from coordinated activity across nearly all neuronal cell types and essential for memory consolidation. In parallel, accumulating work points to a key role for non-neuronal cells—particularly astrocytes—in the regulation of neuronal activity across brain circuits. Yet, how astrocytes engage with distinct neuronal cell types in vivo and shape network dynamics remains poorly understood. Here, we address this gap by combining astrocyte calcium fiber-photometry with large-scale chronic electrophysiological recordings and a recently developed classifier-based framework for neuronal cell-type identification. Strikingly, we reveal a strong negative correlation between astrocytic calcium activity and SPW-R occurrence, evident already within single recording sessions and even within individual astrocytic calcium events. This negative correlation was strongest during NREM sleep, ruling out behavioural or locomotor confounds. Both CCK/Id2 and a subset of Sst interneurons remain selectively active during astrocytic calcium events, suggesting a cell-type-specific engagement of inhibitory circuits associated with reduced network excitability and astrocyte-controlled suppression of SPW-R expression. Future experiments will test the sufficiency and necessity of astrocytic control over hippocampal SPW-Rs by optogenetically activating astrocytic networks using melanopsin. Overall, our results identify a functional role for astrocytes in SPW-R dynamics, and advance our understanding of how astrocytes regulate circuit activity and memory.

42. Are task rules encoded in global cortical dynamics? Insights from a multisensory selection task.

Ayaz Asli [1,2,3,4], Gerova Mihaela[1,2,3], Wei Yu-ting[1,2,3], Bonin Vincent. [1,2,3]
1 KU Leuven, Leuven, Belgium;
2 VIB-KU Leuven Center for Neuroscience, Leuven, Belgium
3 Leuven Brain Institute, Leuven, Belgium
4 VIB-Technology, Neurophysiology Expertise Unit, Leuven, Belgium

It has been proposed that cortical networks dynamically gate behaviorally relevant sensory information to support context-dependent sensory processing. While contextual modulation within individual sensory cortices has been demonstrated, direct evidence for goal-directed selective flow of information at the level of global cortical networks remains limited. To address this question, we designed a multisensory task in which behavioral context can be varied independently of sensory input and motor output, while observing cortex-wide activity patterns using widefield calcium imaging.

In our multimodal sensory selection task, head-restrained mice are presented with identical visual and tactile stimuli under different task rules, defined by reward contingency for one sensory modality at a time. For specific stimulus conditions, this task design isolates task rule as the primary changing variable while holding sensory input and motor output constant, allowing direct comparison of rule-dependent cortical activity. Mice reliably acquire the task and adapt to rule switches, with stable licking patterns across contexts and modulation of pupil size and whisking by reward contingency.

We recorded cortex-wide neural activity using one-photon calcium imaging during passive sensory stimulation or during visual or tactile detection rules. Comparisons of stimulus-evoked activity revealed robust differences between passive stimulation and task engagement, including widespread recruitment of frontal cortical regions during task performance. However, these global activity measures showed limited sensitivity to the task rule. As previous studies demonstrated, behavioral variables are major drivers of cortical activity. We are currently focusing on analyses that aim to disentangle motor-, and decision-related cortical activity patterns from sensory-driven activity to determine how task rules modulate sensory information flow across distributed cortical networks.

43. Medial prefrontal cortex inhibition alters evidence-dependent switching during foraging.

C. Barezzi[1], E. Condon[1], M. Molano-Mazón[2], J. de la Rocha[1], C. Sindreu[1,3]
1 Institut d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS), Barcelona, Spain
2 Universitat Politècnica de Catalunya (UPC), Barcelona, Spain
3 Centro de Investigación Biomédica en Red en Enfermedades Raras (CIBERER), Spain

During natural foraging, animals continuously adapt decisions based on past actions, outcomes, and changing reward contingencies. Previous work has shown that action values (AVs)—internal estimates of expected reward—are stably represented across trials in the medial prefrontal cortex (mPFC), but the causal role of mPFC in adaptive foraging decisions remains unclear.

Here, we investigated mPFC involvement in adaptive choice behavior in mice performing an automated two-armed bandit task with block-wise changes in reward probabilities and variable inter-trial intervals (0–30 s). To causally probe mPFC function, we expressed the inhibitory opsin ACR2 in pyramidal neurons and activated it with 473 nm light during the ITI in 20% of trials.

Behavioral analyses showed that rewards obtained from the alternative option, rather than omission of reward from the chosen option, promoted switching. Model-free analyses revealed that photoinhibition increased switching rates and reduced choice accuracy; notably, switches under photoinhibition were more often followed by unrewarded outcomes.

We then estimated the probability of switching (P(switch)) using a generalized linear model (GLM) including cumulative rewards and errors for both chosen and alternative options. The model log-odds were defined as Switching Evidence (SE), summarizing the total evidence favoring a switch. GLM analyses revealed that photoinhibition reduced the sensitivity of choices to SE: negative SE exerted a weaker stabilizing effect, leading to excessive switching.

Together, these results indicate that mPFC activity shapes how accumulated evidence is used to guide adaptive choice updating, and that its disruption leads to maladaptive switching behavior.

44. Arousal dynamically reshapes the dimensionality of visual cortical populations during continuous stimulation.

Micheli, Rompani.
EMBL Rome

Neural activity in sensory cortex is strongly influenced by internal brain states such as arousal and behavioural engagement. Previous work has shown that internal states fluctuations modulate firing rates, variability, and correlations in cortical populations, with important consequences for sensory encoding. However, most studies have relied on brief, trial-based stimulation paradigms, studying the effect of arousal on trial-averaged stimulus responses and residuals. Here, we investigate how arousal and locomotion modulate population-level activity in primary visual cortex (V1) under sustained visual stimulation. We performed two-photon calcium imaging across cortical layers in mouse V1 while presenting extended epochs of continuous visual stimuli with varying complexity, from simple moving patterns, to naturalistic movies. Rather than focusing on trial-averaged responses, we analysed how arousal dynamically reshapes the geometry and dimensionality of continuous population activity. As expected, we find that arousal globally increases neural activity across layers through a low-dimensional shared activity mode, but exerts distinct, layer-specific effects on the structure of the population activity. During low-complexity stimulation, arousal has only marginal effects on the structure of L4 populations, with most of the neural variance being explained by the simple stimulus dynamics. In contrast, in superficial layers (L2/3), the shared activity mode induced by arousal is much stronger and captures a large fraction of neural variance, leading – at the seconds timescale - to a significant reduction of population dimensionality despite increased firing rates. When stimulus complexity is increased using naturalistic movies, arousal leads to a more dramatic dimensionality reduction in both layers, indicating an interaction between sensory richness and state-dependent modulation of the population geometry. Moreover, instead of considering arousal merely as a contextual modulator of stimulus coding, we emphasize how its global effect on the population activity could actively encode for higher-level stimulus properties, such as stimulus richness and novelty. Interestingly, this process allows for simultaneous representations of lower-level stimulus features at milliseconds timescale and of higher-level features at the seconds timescale. These findings show how the effect of internal states on cortical populations varies across cortical layers and stimulus complexity, and highlight the importance of continuous, single-trial stimulation paradigms for understanding how the brain might implement hierarchical representations at different functional and temporal scales.

45. Threat Learning Recruits an Evolutionarily Conserved Amygdala Circuit.

Noëmie Mermet-Joret*[1], Milad Nazari*[1], Andreas T. Pommer[1], Sanaz Ansarifar[1], Joana Silva Luz[1], Anne-Katrine Vestergaard[1], Sadegh Nabavi[1]

1. Department of Molecular Biology and Genetics, Aarhus University, Denmark; Center for Proteins in Memory - PROMEMO, Danish National Research Foundation, Denmark

Certain sensory stimuli trigger defensive responses because they are learnt, through repeated experiences, to predict a threat. Some other stimuli are perceived as a threat upon the first exposure; they are innately aversive. The circuit processing innate threat is prewired while that processing learned threat is sculpted through synaptic plasticity. However, both forms of threat are processed in the basolateral amygdala (BLA). This raises the possibility that learning new threats engage an evolutionary conserved prewired circuit in the BLA.

To test this, we examined three key components within the BLA in mice: excitatory neurons, inhibitory neurons, and neuromodulator release; during exposure to an innate visual threat (looming shadow) and throughout learning and recall of an auditory threat (Pavlovian fear conditioning).

Our single-cell–resolution imaging of excitatory neurons revealed that learning-related plasticity was selectively expressed in neurons originally activated by the innate aversive stimulus. This suggests that the neurons processing innate aversive cues are recruited for the acquisition and recall of aversive cues.

Next, we performed fiber photometry from Vasoactive-intestinal-peptide (VIP)- and Somatostatine (SST)- interneurons. We found that while VIP interneurons were activated by the looming shadow, SST interneurons were strongly suppressed. This suggests that the looming-induced defensive responses are triggered through disinhibition of the principal neurons. During fear conditioning, VIP and SST interneurons again exhibited distinct but complementary dynamics, suggesting that the same inhibitory motif is used in threat learning. Finally, we found that innate and learned aversive stimuli -but not a neutral stimulus—trigger the immediate release of norepinephrine (NE) in the amygdala. This suggests that NE reduces the threshold for activating the circuits processing innate as well as learned threats.

Together, our data support the hypothesis that threat learning relies on the prewired circuit processing innate threats.

46. Cellular Mechanisms Underlying Discontiguous Associative Learning in the Medial Prefrontal Cortex.

Ansarifar, Sanaz, Nazari, Milad, Mermet-Joret, Noëmie, Nabavi, Sadegh
Department of Molecular Biology and Genetics, Aarhus University, Aarhus, Denmark

The ability to associate events separated in time is essential for learning, yet it poses a fundamental challenge to dominant models that require near-simultaneous activity for successful associative learning. A leading hypothesis proposes that persistent activity in the medial prefrontal cortex (mPFC) maintains stimulus representations across temporal gaps, enabling the association between separated events. However, how this process is implemented at the cellular and circuit levels remains unclear.

Here, we used a learning paradigm (trace fear conditioning) in which mice learn to associate two events - a tone (conditioned stimulus; CS) and a footshock (unconditioned stimulus; US)- separated by a 20-second interval. We monitored the neuronal activity in the mPFC using miniaturized microscope during learning and memory recall.

We found that neurons responsive to the CS became increasingly time-locked to the tone as learning progressed, resulting in more synchronized activity within the mPFC. Moreover, neurons with long-lasting responses to the US during early learning showed activation during the trace period, potentially bridging the gap between the CS and US. Interestingly, this same set of neurons were more likely to respond to the CS on recall day, indicating their selective recruitment for memory recall. Neurons which were active only during the US, on the other hand, were not likely to responds to the CS on recall day. Furthermore, population activity analyses reveal higher correlation between neuronal activity during the CS on recall day and post-US period on early trials of conditioning session.

Next, we performed fiber photometry from Parvalbumin (PV)- and Somatostatin (SST)- interneurons. We found suppressed activity of PV neurons was strongly correlated with fear expression, whereas SST response to both CS and US increased over training. This suggests complementary roles between the two inhibitory neurons: PV cells contribute to fear expression, while SST cells contribute to learning and memory formation.

Overall, this work provides mechanistic insight for cellular implementation of the discontiguous associative learning.

47. Dimensionality and Geometry of Object Coding Across the Mouse Visual Hierarchy.

Paschalidis Christos [1,2], Aliprantis Stamatis [1,2], Diamantaki Maria [2], Froudarakis Emmanouil [1,2]
1. Department of Medicine, University of Crete, Heraklion, Greece;
2. Institute of Molecular Biology and Biotechnology, FORTH, Heraklion, Greece

Object recognition requires the visual system to construct population-level representations that are robust to large variations in sensory input. A long-standing hypothesis is that such invariance emerges through transformations of neural population geometry along the visual cortical hierarchy. Despite extensive study, there is still no consensus on the dimensionality of visual cortical representations and whether geometric properties identified in primary visual cortex generalize across higher visual areas. Here, we investigate the dimensionality and geometric organization of population activity across the mouse visual cortex using large-scale two-photon calcium imaging. We analyzed simultaneous recordings from head-fixed mice while viewing three-dimensional moving objects under varying stimulus conditions. We extracted the neural activity from the primary visual cortex (V1) and 3 higher visual areas of the mouse, LM, AL & LI. Using linear analyses of population activity, we find that responses in all visual areas exhibit high apparent dimensionality, with the number of dimensions required to explain population variance scaling with the size of the recorded neural population. Importantly, this high dimensionality is stable across repeated subsampling of neurons, suggesting that it reflects genuine structure rather than noise or sampling artifacts. To test whether this high-dimensional variance reflects a lower-dimensional nonlinear structure, we estimated the intrinsic dimensionality, which is the minimum number of parameters to approximate the neural space. Intrinsic dimensionality was substantially lower than its linear counterparts, suggesting a low-dimensional manifold structure of the neural code. Comparisons across visual areas reveal systematic differences in how variance is distributed across population activity dimensions, with areas such as LI and AL concentrating more variance into their primary dimensions, relative to V1. To interpret the functional relevance of this geometry, we leveraged the behavioral state as a natural modulation of cortical processing. In particular, during locomotion, object-identity decoding improves across all visual areas, indicating that active behavioral states enhance the alignment of population activity with task-relevant dimensions. Specifically, we compute a separation index that evaluates linear separability between object manifolds in the neural space, revealing that during locomotion decoding is improved by pushing object representations farther apart. Together these results support a geometric view of object representations in which visual cortical population activity remains high-dimensional, yet exhibits area-specific and state-dependent organization. Thus it ultimately constrains how object information is embedded and accessed across the visual hierarchy.

48. Sleep fragmentation differentially alters hippocampal network dynamics and memory in wild-type and APP-PS1 mice.

Rigamonti, Giulia [1,2], Molina Paya, Francisco Javier [1], Cabral Pereira, Giselda [2], Gascon Lopez, Irene [2], Tirado, Manuel [1], Abad Perez, Pablo [3], Brotons Mas, Jorge [1,2], Sanchez Mut, Jose Vicente [2]
1. Universidad CEU Cardenal Herrera, Departamento de Medicina y Cirugía, Alfara del Patriarca, Spain
2. Instituto Neurociencias Alicante CSIC-UMH, Sant Joan d'Alacant, Spain
3. Hospital del Mar Research Institute, Barcellona, Spain

Dementia in Alzheimer's disease (AD) cannot be explained solely by beta-amyloid and TAU pathology. Additional AD-associated factors, such as sleep hygiene, significantly influence disease outcome. Here, we hypothesize that sleep fragmentation (SF) contributes to disease progression by impairing sleep-dependent network processes involved in memory formation and consolidation.

To test this hypothesis, we compared wild-type (WT) and APP-PS1 mice using hippocampal electrophysiology in freely-moving animals during sleep and memory processing, combined with behavioral assessment in the open field and novel object recognition tasks, both before and after a 5-day SF protocol.

At baseline, APP-PS1 mice exhibited fragmented sleep architecture, together with memory deficits and hippocampal network dysfunction characterized by hyperactivity, hypersynchronization, altered oscillatory coupling - particularly during sharp-wave ripple events associated with memory consolidation - and reduced spatial coding efficiency of place cells.

SF impaired memory in both groups, with a larger decline in WT mice. These behavioral deficits were accompanied by genotype-specific electrophysiological changes: WT mice showed degraded spatial coding in place cells, whereas APP-PS1 mice exhibited a further exacerbation of hippocampal hyperactivity and excitation/inhibition imbalance.

These findings suggest that cognitive deficits in APP-PS1 mice reflect chronic alterations in sleep quality and hippocampal circuit dynamics, where increased neuronal activity and synchronization interfere with information consolidation. Moreover, sleep fragmentation differentially affects memory depending on baseline circuit state, inducing dementia-like dysfunction in healthy brains and accelerating pathological processes in AD animals.

Together, these results support the role of sleep disruption as a key modulator of hippocampal circuit's vulnerability to AD progression.

49. Prefrontal Cortex Feedback Reshapes the Representation and Routing of Visual Information.

Ährlund-Richter, **Sofie**[1], Yuma Osako, Yuma[1], Jenks, Kyle R.[1], Heller, Gregg R.[2], Sooter, Sam[2], Sur, Mriganka[1,2].

1 The Picower Institute for Learning and Memory, Massachusetts Institute of Technology, Cambridge, MA, USA.

2 Department of Brain and Cognitive Sciences, Massachusetts Institute of Technology, Cambridge, MA, USA.

How does the brain determine which sensory signals are behaviorally relevant, and how are these signals routed across cortical networks? Top-down feedback from the prefrontal cortex (PFC) has long been proposed to play a central role in shaping sensory processing, yet the circuit-level mechanisms by which distinct prefrontal pathways modulate sensory representations remain poorly understood.

Here we investigate how anatomically and functionally distinct prefrontal subregions influence visual processing in primary visual cortex (VISp). Using a combination of axonal tracing, two-photon calcium imaging, and projection-specific chemogenetic perturbations in behaving mice, we show that inputs from the anterior cingulate cortex (ACA) and orbitofrontal cortex (ORB) exert dissociable and context-dependent effects on visual responses. ACA feedback enhances visual encoding in a behavioral-state-dependent manner, selectively amplifying sensory representations during arousal. In contrast, ORB feedback preferentially modulates arousal- and movement-related activity in VISp and can suppress the encoding of high- contrast visual stimuli. These findings demonstrate that prefrontal feedback is not a uniform gain signal but instead operates through modular pathways that differentially shape sensory representations according to internal state and behavioral context.

Building on these results, we are developing next-generation in vivo approaches to uncover the cell-type-specific logic underlying cortical communication. By combining spectral unmixing–based two-photon imaging, projection-defined labeling, and targeted perturbations, we can simultaneously image multiple, defined VISp neuron populations distinguished by their long-range outputs. This strategy enables direct observation of how prefrontal feedback redistributes visual information across parallel cortical output channels in real time.

Together, this work reveals how top-down control dynamically restructures sensory representations and routing in the cortex. By defining circuit principles of prefrontal modulation, these findings provide a framework for understanding how disruptions in feedback control may contribute to altered perception and cognition in neurodevelopmental disorders.

50. Social context selectively enhances binge alcohol consumption and weakens activation of an inhibitory mOFC ensemble.

Mas-Gómez, **Fuensanta**, Vélez-González,Helden, Leroy,Félix, Giménez-Gómez, Pablo.
Institute of Neuroscience of Alicante (IN)

Binge drinking is highly prevalent among adolescents and young adults and typically occurs in social settings rather than isolation. Despite strong epidemiological evidence showing that peer context is a major driver of early alcohol use, most preclinical studies investigate alcohol intake in individually housed animals. This gap limits our understanding of how social environment reshapes the neural circuits that regulate binge drinking.

We previously identified a neuronal ensemble in the medial orbitofrontal cortex (mOFC) that is activated during binge alcohol intoxication and functions as a brake on excessive drinking. Optogenetic manipulation demonstrated that activation of this ensemble suppresses alcohol intake, whereas its inhibition increases consumption. However, whether social context alters this inhibitory circuit remains unknown.

Here, using the Drinking-in-the-Dark (DID) paradigm, we compared alcohol intake in isolated and group-housed mice. Group housing significantly increased alcohol consumption in both males and females relative to isolated conditions. This effect was replicated in TRAP2xAi9 mice, enabling future activity-dependent tagging of neuronal ensembles engaged during social drinking. Importantly, the social facilitation effect was selective for alcohol, as no differences were observed in water or saccharine consumption.

At the neural level, mice drinking in social groups exhibited reduced Fos expression in the mOFC compared to isolated drinkers, suggesting that social context attenuates activation of the inhibitory mOFC ensemble.

Together, these findings indicate that peer environment selectively amplifies binge drinking while weakening cortical inhibitory control, providing a circuit-level framework for socially facilitated alcohol consumption.

51. Neuroanatomical and Functional Characterization of the Mouse Indusium Griseum.

Vélez González, **Helden**; Giménez Gómez, Pablo; Leroy, Félix.
Instituto de Neurociencias de Alicante, Alicante, Spain

The indusium griseum (IG) is a brain structure composed of two narrow longitudinal bilateral strips that run above the corpus callosum (CC) in its rostrocaudal length. The IG is critical during development for the formation of the CC and connects with the hippocampus, cingulate cortex and septal region. However, its function in adult mice remains unknown. We hypothesized that it might be involved in social discrimination, since it is linked with key hub regions for this type of behavior. To test this, we utilized Avpr1b-Cre mice of both sexes to specifically target the IG, which exhibits high Avpr1b expression. We employed anterograde and retrograde viral tracing to map its connectivity and used inhibitory DREADDs to assess the behavioral impact of silencing Avpr1b+ IG neurons in locomotion, anxiety, feeding, olfaction and social behaviors. We found that the IG receives primary inputs from the anterior cingulate, motor, piriform, entorhinal, and retrosplenial cortices, while projecting mainly to the fasciola cinerea and the septohippocampal nucleus. Behavioral assays showed that IG inhibition did not affect locomotion, anxiety, feeding, or olfaction. However, it significantly impaired the familiarization process during social novelty and repetitive presentation tests. Our preliminary results suggest that the IG acts as a functional hub, integrating cortical signals and social cues to mediate social familiarization in mice.

52. Unveiling spatial metabolic alterations driving disease progression in progressive multiple sclerosis.

Esposito, Riccardo[1]; Finardi, Annamaria[2]; Malik, Zulkifal[1]; Amenta, Alessia[1]; Furlan, Roberto[2]; Martano, Giuseppe[3]; Bifari, Francesco[1]

1) Laboratory of Cell Metabolism and Regenerative Medicine, Department of Medical Biotechnology and Translational Medicine, University of Milan, Milan, Italy; 2) Clinical Neuroimmunology Unit, Institute of Experimental Neurology (INSpe), Division of Neuroscience, San Raffaele Scientific Institute, Milan, Italy; 3) CNR Institute of Neuroscience, Milan, Italy.

Progressive multiple sclerosis (P-MS) represents a major challenge due to the complexity of its pathogenesis and the lack of effective therapies. P-MS is characterized by chronic neuroinflammation, demyelination, neurodegeneration, and profound metabolic alterations in both neuronal and immune cells. However, the contribution of these metabolic deficits to disease progression remains poorly understood.

To address this gap, we employed the chronic experimental autoimmune encephalomyelitis (cEAE) mouse model, which recapitulates key features of P-MS, and applied high resolution spatial mass spectrometry (SMS) to map metabolic alterations across brain regions. This multimodal approach integrates spatial metabolomics with computational clustering and dimensionality reduction analyses. UMAP clustering revealed distinct metabolic profiles in wild type and cEAE mice, indicating pronounced metabolic disorganization in cEAE. Spatial cluster analysis delineated white and grey matter regions and identified a white matter specific metabolic cluster that was lost in cEAE. Enrichment and spatial correlation analyses revealed marked alterations in alanine, aspartate, and glutamate metabolism pathways linked to the Krebs cycle, excitotoxicity, and oxidative stress together with increased biosynthesis of arachidonic acid and unsaturated fatty acids, consistent with persistent inflammatory signaling.

To counteract these alterations, we tested the effect of metabolic reprogramming therapy in cEAE mice based on the administration of metabolic precursors, including essential amino acids, Krebs cycle intermediates, and co-factors. SMS analysis showed that treated mice exhibited an intermediate metabolic phenotype between wild type and cEAE animals, with partial restoration of amino acid and lipid related metabolic pathways.

Overall, this study demonstrates that spatially resolved metabolic mapping combined with computational analyses can help clarify mechanisms underlying disease progression in P- MS. These findings support targeted metabolic modulation as a promising approach to restore neuronal function and reduce chronic neuroinflammation and neurodegeneration.

53. Cortico-striatal neurons in the anterior lateral motor cortex are necessary to drive learning of sensory-guided decisions

Lenzi, Irene [1,2] , Nojavan Lahiji, Nilufar [1,2] , Despatin, Alice [1,2] , Nesseler, Moritz [2] , Spehr, Marc [2] , Kampa, Björn [1,2] , Musall, Simon [1,2,3]

Forschungszentrum Jülich, Jülich, Germany. 2. RWTH Aachen University, Aachen, Germany. 3. Institute of Experimental Epileptology and Cognition Research, University of Bonn, Bonn, Germany

The anterior lateral motor cortex (ALM) is critical for sensory decision-making, relying on long-range projections to subcortical regions, particularly the striatum via cortico- striatal (CStr) neurons. This pathway may support forming new sensorimotor associations, but its role during learning is unclear. At first, we anatomically mapped ALM terminals in the striatum, finding a conserved anteroposterior gradient across ipsilateral and contralateral projections. Then, mice were trained in tasks of increasing cognitive demand while we tracked learning-related changes in ALM axonal activity in the striatum using fiber photometry. Neural and motor activity increased with learning. A GLM isolated task- related signals, showing heightened activity during early training, in both stimulus and choice periods, supporting CStr involvement in forming sensorimotor associations. Synaptic silencing of ALM CStr synapses confirmed their role during early task performance. To assess the functional specificity of the CStr pathway, we at first triple labeled ALM projections to the striatum, thalamus, and superior colliculus with a retrograde approach, revealing neurons uniquely projecting to each target. Fiber photometry of thalamic and collicular projections showed increased pre-stimulus and choice-related activity during late training, consistent with a functional role after associations stabilized. Synaptic inhibition of these pathways reduced performance in the expert phase. Because of the within and across layer variability in neuronal activity, we then retrogradely labelled ALM CStr neurons and performed multi-layer two-photon imaging during active behavior, which revealed layer- and population-specific differences in choice selectivity. Overall, we confirmed that task learning induces long-lasting changes in ALM choice-related circuits, with distinct subcortical pathways contributing at different learning stages and distinct CStr projections driving early learning of sensory-driven decisions.

54. The role of somatosensory information in a bilateral reference formation.

Jorge Matas-Teruel, Alicia Alonso-Andrés, Ramón Reig.
Instituto de Neurociencias CSIC-UMH

The whisker system is used by rodents to explore the environment, sensing the presence of objects along their path, for example, providing information about obstacles or to signal the entrance of a shelter. Contrasting whiskers information from the two sides of the snout can thus be crucial to accomplish such behavior. Sensory information collected through vibrissa is relied to the contralateral barrel cortex (BC) following a somatotopical organization; in fact, it is characterized by being a highly lateralized system. However, functional and anatomical differences have been described between the subregion of the BC associated to whiskers in distinct rows. Previous work in our group has shown that the barrels processing the input of medially located whisker follicles have a more relevant role in callosal-mediated interhemispheric communication than those located more laterally (Montanari et al., Sci. Adv. 2023). Therefore, they are a candidate to be involved in actions that require bilateral integration between hemispheres. In humans, alterations in the corpus callosum (CC) connecting visual areas proved to have an effect on depth perception, demonstrating that callosal inputs have a functional role. However, the bilateral function of the rodents whisker systems remaining elusive. Based on our previous results, we hypothesize that in the case of the whisker system, highly interhemispherically connected areas could be associated to the generation of a bilateral reference centered to the midline of the face in the context of object localization and exploration. To interrogate this hypothesis, we have designed a behavioral paradigm in which freely moving mice approach a water spout on a location they have been previously trained to locate. This task enables the use of different perturbations of the whisker system and the measurement of the time spent in successfully reaching and drinking from the water source. Moreover, by using a high-speed infrared vision camera, it is possible to track different movement patterns that might be reflecting the impact of the missing whiskers input. Our preliminary results show an impairment on the task performance when blocking medial whiskers sensory transmission, in addition to altered movement patterns regarding landmarks in the arena. All in all, we are characterizing the differential contribution of the distinct rows of the whisker system to the generation of a bilateral spatial reference centered in the midline.

55. Genetic dissection of GluN3A-mediated stress resilience.

Rémy Verhaeghe, Carmen García-Lira, Luis García-Rabaneda, Luis F. Callado, Jose Javier Meana, William C Wetsel, Isabel Perez-Otaño

1. Instituto de Neurociencias (CSIC-UMH), San Juan de Alicante 03550, Spain; 2. Department of Pharmacology, University of the Basque Country, Leioa, Bizkaia, Spain; 3. Department of Psychiatry and Behavioral Sciences, Duke University, United-States

Chronic stress affects brain structure and function and is a major risk factor for neuropsychiatric disorders including post-traumatic stress disorder, anxiety and major depressive disorder (MDD). A principal neural adaptation is a long-lasting alteration in synaptic connectivity that is thought to be caused by impaired local protein translation. The mammalian target of rapamycin complex 1 (mTORC1) is a key determinant of translation, and current efforts are centered in therapeutics that foster mTORC1 activation—a prime example is the NMDAR antagonist ketamine. Recent work implicates the GluN3A non-conventional NMDA receptor subunit,as a key regulator of mTORC1 signaling at synaptic loci1. Yet to date efforts have focused on targeting classical NMDA receptors, and GluN3-NMDARs have been overlooked despite selective expression in brain regions critical for stress regulation such as the prefrontal cortex, ventral hippocampus or amygdala2. Using chronic stress models, we now report that deletion of GluN3A in male and female mice confers stress resistance tested in a variety of paradigms (light-dark box, elevated plus maze and tail suspension test). Stress resistance of GluN3A KO mice was associated with stronger mTORC1 activation levels in basal conditions and upon chronic stress, as shown by enhanced or reduced phosphorylation of the mTORC1 downstream targets S6 and eEF2. Conversely, overexpression of GluN3a increased anxiety-like and depressive-like behaviors. Using conditional knockout GluN3A mice, we identified excitatory neurons in prefrontal cortex as the locus for GluN3A modulation of stress responses. At the mechanistic level, we found that GluN3A and mTORC1 machinery are altered in MDD patients and chronic stressed mice models, suggesting that GluN3a might be a new promising therapeutical target for chronic stress-related disorders.

[1] Conde-Dusman et al eLife 2021; [2] Murillo et al Cerebral Cortex 2021

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56. GluN3A deletion disrupts region- and laminar-specific targeting of interhemispheric callosal axons.

B. Corral-Sanchez , I. Perez Otaño, O. Crawley , A.I. Navarro
Instituto de neurociencias, San Juan de Alicante, Spain

Callosal projections are long-range axonal projections that connect the two cortical hemispheres via the corpus callosum, allowing bilateral integration of sensory information. Callosal axons originate mainly from layer (L) 2/3 pyramidal neurons in primary sensory areas and project to homotypic regions of the contralateral hemisphere. Accurate information transfer relies on a highly stereotyped region and layer-specific axonal pattern, targeting distinct dendritic domains of contralateral L2/3 neurons. In mouse somatosensory cortex such precise innervation is established during the second and third postnatal weeks but the molecular determinants are largely unknown. Using in utero electroporation of fluorescent reporters to label L2/3 neurons, we show that loss-of-function of Grin3a (gene encoding the non-conventional NMDA receptor subunit GluN3A) disrupts region and layer-specific callosal axon targeting without altering early axonal navigation steps. Rather than concentrating in the border between primary/secondary somatosensory cortex (S1/S2), callosal axons in GluN3A knockouts form an ectopic second column laterally in S2. Within the S1/S2 border, axonal arbors fail to innervate their normal destinations in L1 and are shifted towards inner regions of L2/3. Conditional loss of function experiments, together with altered apical dendritic branching in Grin3a knockouts, suggest that the dendritic architecture of postsynaptic L2/3 neurons guides callosal axon patterns during critical periods of circuit maturation and implicate GluN3A NMDA receptor subunits in determining the timing and specificity of this process. Finally, we identify GluN3A modulation of Kcna1 and Crmp4 expression as downstream targets, linking their tempo of expression with the spatial precision of dendritic arbor development.

57. A brainstem substrate for coordinating body, face, and eye movements during orienting.

Alexis d'Humières, Guillaume Le Goc, Antoine Lesage, Nella Znaor, Giovanni Usseglio, Julien Bouvier
University Paris-Saclay, CNRS, Paris-Saclay Institute of Neuroscience, France

Most motor behaviors can be broken down into "elementary" actions, each involving a restricted set of muscle groups. The brainstem reticular formation (RF) is well-positioned to control such actions at both the spinal (trunk, limbs,…) and cranial (orofacial, ocular,…) levels. Yet, the specific roles of its diverse neuronal populations remain poorly understood. We recently showed that V2a neurons, a glutamatergic subset in the RF, impose limb and trunk asymmetries, thereby engaging locomotor turning. These actions were attributed to spinal projections of V2a neurons of the gigantocellular nucleus (Gi). We question here the possibility that V2a neurons, in the Gi and/or in adjacent brainstem regions, could control additional actions pertinent for orienting, including at the cranial level.
We investigate this by combining, in the mouse, cell-type specific manipulations, connectomics, and optogenetics. First, an efferent connectivity mapping revealed that V2a Gi neurons make abundant synaptic contacts in the brainstem and midbrain, with the most contacted regions being specific sets of cranial motoneurons. Functionally, activating V2a Gi neurons indeed reliably evokes orofacial movements, compatible with a premotor connectivity. Second, we found that the activation of the spinally-projecting V2a Gi subset only reproduces a fraction of these orofacial movements, suggesting the existence of exclusively locally projecting V2a neurons. Third, investigating V2a neurons located beyond the Gi revealed that the more rostral ones (pontine RF) can additionally mobilize specific ocular movements, suggesting a rostro-caudal specialization of this cell-type. Conversely, the inhibition of rostral V2a neurons perturbs eye movements during a head-fixed optokinetic reflex, placing them as an indispensable output pathway for gaze control.
Together, these results identify V2a reticular neurons as a heterogeneous hub for orienting, in which distinct subtypes control specific elementary actions. This may enable their differential selection and combination during complex behaviors such as orienting."

58. Adaptive changes in oxytocinergic hypothalamic nuclei.

Sonia Amoros Bru, Maria Pilar Madrigal, Sandra Jurado
Instituto de neurociencias, Alicante, Spain

Oxytocin (OXT) is a crucial neuropeptide primarily synthesized in hypothalamic nuclei, where it regulates parental behaviors, social interactions, and pro-social neuromodulation, playing a vital role in establishing social bonds. The proper development of oxytocinergic circuits supports these functions, influencing sex-specific and context-dependent social behaviors critical for survival and adaptation to changing environments. However, the structural and functional changes in these circuits during key life stages, such as motherhood or aging, remain insufficiently explored. To address this, we employed the iDISCO+ tissue clearing method combined with light-sheet fluorescence microscopy to generate a three-dimensional reconstruction of the oxytocinergic system in adult and aged mouse brains. Our analysis revealed an increase in the number of oxytocin-positive (OXT+) cells in the supraoptic nucleus (SON) and in the anterodorsal preoptic nucleus (ADPN) of postpartum females, whereas no significant changes were observed in other major oxytocinergic regions, such as the paraventricular nucleus (PVN). In aged mice, we detected a reduction in OXT+ cells within the bed nucleus of the stria terminalis (BNST) of females and the ADPN of males. Furthermore, these nuclei exhibited a decreased proportion of magnocellular OXT+ cells, a distinct oxytocinergic neuronal subtype. Our findings suggest that further modifications may occur under pathological aging conditions. These results provide new insights into the plasticity and dynamic, sex- and region-specific changes in oxytocinergic circuits throughout motherhood, and both natural and pathological aging.

59. Plastic properties of hypothalamic oxytocinergic neurons.

Maria Royo, JJ Ramirez-Franco, Sandra Jurado
Instituto de neurociencias, Alicante, Spain.

Oxytocin (OXT) is a powerful neuromodulator of brain function orchestrating adaptive responses to life events as remarkable as parturition and lactation. Furthermore, OXT is considered a pro-social neuropeptide decreasing social anxiety and promoting social recognition and social memory. OXT is a hormone and a neuropeptide, mainly synthetized in the supraoptic (SON) and the paraventricular (PVN) nuclei. We will focus our work on the PVN neurons, which extend their projections to multiple brain areas, where OXT modulates basal synaptic properties and synaptic plasticity. However, very little is known about the functional microcircuit connectivity within the hypothalamic oxytocinergic nuclei. The most recent evidence suggests that oxytocinergic neurons in the PVN undergo substantial plastic changes, enabling them to modulate their connections in response to environmental variations. Understanding of these changes is essential in addressing the role of the oxytocinergic system in normal physiological functions.

60. The role of hippocampal inhibition in spatial memory encoding.

Elena Pérez-Montoyo*, José María Caramés*, Raquel Garcia-Hernandez, Santiago Canals# and Encarni Marcos#
co-first; #co-corresponding
Instituto de neurociencias, Alicante, Spain

The dentate gyrus (DG) plays a key role in spatial memory discrimination and in coordinating dispersed neuronal populations during memory formation. The excitatory- inhibitory balance within the DG is thought to be critical for this process. Here, we show that inactivation of parvalbumin (PV) interneurons enhances spatial discrimination. Using a computational model, we predict that such improvement in spatial discrimination impairs memory stability. This prediction is confirmed by experimental work in mice, in which animals were presented with multiple contexts while PV neuron activity was manipulated. During testing, animals with intact DG activity remembered the spatial positions of objects better than those with inactivated PV neurons. Our results highlight the importance of the excitatory-inhibitory balance within the DG for maintaining memory discrimination and stability.

61. Brain-Wide Network Dynamics: An Assembly-Level Perspective

Fabrizio Londei [1,2], Giulia Arena [2,3,4], Lorenzo Ferrucci [3], Francesco Siano [2,3], Francesco Ceccarelli [3], Encarni Marcos [1], Aldo Genovesio [3,5]

1) Instituto de Neurociencias, Consejo Superior de Investigaciones Científicas and Universidad Miguel Hernández, Sant Joan d'Alacant, Alicante, Spain. 2) Behavioral Neuroscience PhD Program, Sapienza University, Rome, Italy. 3) Department of Physiology and Pharmacology, Sapienza University of Rome, Rome, Italy. 4) Institute of Biochemistry and Cell Biology (IBBC), National Research Council of Italy (CNR), Monterotondo Scalo, Roma, Italy. 5) Department of Pharmaceutical Sciences, University of Piemonte Orientale, Novara, Italy.

Efficient cooperation between brain areas requires a dynamic balance between the segregation of region- specific functional roles and information broadcasting. To understand how the brain achieves this coordination, we must move beyond macro-scale imaging and examine the electrical activity of single neurons. In this study, we analyzed spiking activity from electrophysiological recordings using the large- scale public dataset by Steinmetz and colleagues. We applied a statistical algorithm to identify cell assemblies, which are groups of neurons that transiently and repeatedly co-activate to form the fundamental computational units of the brain. We initially tested our framework on a highly interconnected subthalamic region, the Zona Incerta, to map its specific network dynamics. Building on these results, we then expanded our analysis across the dataset to investigate brain-wide interactions. Rather than limiting our scope to simple pairs of neurons, we sought to identify more complex higher-order coordinative structures. Specifically, we focused on "loop-like motifs", defined as triplets of neurons distributed across two different areas that model a reentrant flow of information. We observed that this reentrant communication operates largely independently of basic pairwise coordination. Our primary finding highlights the crucial role of hub neurons in this process. Specifically, our analysis revealed that external hubs (neurons exhibiting high coordinative connectivity with other brain areas) are consistently and significantly embedded within loop-like motifs across all analyzed regions. Importantly, this embedding is not merely a byproduct of general cellular hyperconnectivity. Indeed, the relationship between these motifs and internal hubs (neurons with high local intra-areal connectivity) proved to be highly area-specific rather than universal. We hypothesize that this robust and specific association with external hubs may serve as a distinct signature of inter-regional integration, suggesting that loop-like motifs could provide a core architectural mechanism for broadcasting and combining information from diverse circuits across the brain.