Fundação Champalimaud

About: Founded in 2004, the Champalimaud Foundation (CF) is a private, non-profit organization dedicated to making advances in biomedical science. The core focus of the foundation is to achieve breakthroughs in neurosciecnce and cancer research. Champalimaud Research started with the Champalimaud Neuroscience Programme, created in 2007, as a basic research team wit the broad aim of understanding brain function through integrative biological approaches. Currently, the Neuroscience team is composed of 17 main research groups, plus research associates and adjunct investigators, who study diverse topics in neuroscience using advanced, cutting edge techniques.

Intern responsibilities: IIP interns will be able to choose working in one of the following labs. They should state their preferred lab and project in their personal statement and address the reason for their choices.

- **Dr. Carey Lab**

  <u>Cerebellar contributions to coordinated locomotion in mice</u>
  Gait ataxia, or uncoordinated walking, is one of the most prominent symptoms of cerebellar damage, but the mechanisms through which the cerebellum contributes to coordinated locomotion are not well understood. Both ataxic mouse mutants and the sophisticated genetic tools available for manipulating neural circuits in mice have the potential to help shed light on this problem. However, analyses of mouse gait have typically lacked the kind of detail about the precision and timing of limb movements that would be required for a full analysis of coordination. We have built a custom video tracking system (LocoMouse) for measuring and analysing overground locomotion in freely walking mice. The LocoMouse system automatically detects the position of paws, snout, tail, and body centre in all three spatial dimensions with high spatiotemporal resolution. We have used this system to establish a quantitative framework for coordinated locomotion in mice (Machado et al. 2015). This approach allows us to identify specific, cerebellum-dependent features of locomotor coordination and to probe circuit mechanisms supporting complex, whole-body movements.

  <u>Neural mechanisms of locomotor adaptation</u>
  Locomotor patterns are constantly adapted for changing environments but the neural mechanisms underlying this basic form of learning are not well understood. Locomotor adaptation has been studied in humans using a motorized split-belt treadmill in which the limbs on opposite sides of the body move at different speeds. Subjects adapt to split-belt walking over time by changing spatial and temporal gait parameters, which show negative after- effects in post- adaptation. This type of motor learning is thought to involve the cerebellum, as previous studies have indicated that patients with cerebellar lesions cannot adapt to the perturbation (Morton & Bastian, 2006). However, the circuit mechanisms within the cerebellum that support this adaptation are not known. We have built a split-belt

treadmill for mice and are using it in combination with genetic and electrophysiological tools to investigate the neural basis of locomotor adaptation.

Behavioral state modulation of associative learning in mouse cerebellum
Delay eyeblink conditioning is a relatively simple form of cerebellum-dependent associative learning. Recent work has demonstrated, however, that neither the learned behavior nor its underlying neural circuitry are as simple as once thought. We have recently found that locomotor activity modulates delay eyeblink conditioning through mechanisms that act on the mossy fiber pathway within the cerebellar cortex. These results suggest a novel role for behavioral state modulation in associative learning and provide a potential mechanism through which engaging in movement can improve an individual's ability to learn. Ongoing experiments are investigating the mechanisms and consequences of this modulation.

- **Dr. Chiappe Lab**

Sensory processing and motor actions are intimately linked in many aspects of brain function. Examples include active sensing, goal-directed locomotion and motor learning. We use these behavioral contexts to investigate the underlying operational principles of sensorimotor processing. Our projects are divided in three inter-related lines of research:

Development of behavioral paradigms to study sensorimotor integration
We are currently developing "freely moving" and "tethered" behavioral paradigms in virtual reality-like worlds designed to probe the computational capacities of the fly's brain during visually guided orientation behaviors. These shall form a platform for studying: a) how the fly uses its own movements and the generated visual motion cues to explore an environment, b) how her brain incorporates sensory signals to correct locomotion during orientation towards objects, and c) how do past experiences inform ongoing behavior.
Collaborators: Gonçalo Lopez, José Cruz and Matthieu Pasquet

Identification of neurons and circuits involved in sensorimotor processing
The aim of this project is to understand how components in the circuit are linked and how the activity patterns of neurons arise from their synaptic connectivity. We identify neuronal components of a network using behavioral, physiological and anatomical methods. We then map connectivity among candidate neurons by combining chemical, optical and electrical techniques. Importantly, in the brain of the fruitfly it is possible to systematically identify the same class of neurons across different individuals. This allows investigating variability in synaptic connectivity and circuit function across different flies.

Probing neural processing during sensorimotor tasks
In simultaneous with head-fixed, tethered locomotion, we use electrophysiological and imaging techniques to monitor the activity dynamics of populations of genetically- or anatomically-defined groups of neurons. We apply quantitative analytical tools to correlate neural population activity with the behaviors described above, and to make predictions about the contribution of different groups of neurons to such behaviors. We examine the roles of different groups of neurons in the circuit by precise manipulations of their activity with genetic and optical techniques. These experiments are aimed at defining the functional

logic of the circuitry in the context of a specific behavior. By comparing different visual-motor tasks, our research attempts to identify common principles of visual-motor transformations.

- **Dr. Costa-Silva Lab**

  Our overall goal is to investigate whether heathy tissues, locally and at distance, can contribute to tumor genesis, progression and metastasis, and if extracellular vesicles act as mediators of this process. For that, we are interested in: (i) Novel tumor-associated cells: identify underappreciated non-malignant cellular counterparts (other than fibroblasts, endothelial cells and immune cells) susceptible to tumor-derived messages and with potential role as pro-tumor ancillary cells, (ii) Stroma-tumor communication: identify messages (e.g. extracellular vesicles) derived from non tumor tissues, locally and at distance, that can influence tumor progression, (iii) Phenotypic characterization of extracellular vesicles: by state-of-the-art flow cytometry in combination with detailed molecular characterization, identity extracellular vesicles subpopulations of different cell of origin and/or pathologic background relevant for tumor biology.

- **Dr. Machens Lab**

  How does the brain work? What are the kind of computations carried out by neural systems? We try to address these questions by analyzing recordings of neural activity and constructing mathematical models of neural circuits. Our main goal is to link the activity within various brain areas to a computational theory of animal behavior. We are currently developing methods to summarize the activity of neural populations in useful ways and to compare population activity across areas. In turn, we seek to relate the population activity to behavioral, computational, and mechanistic problems or constraints that organisms are facing. We work in close collaboration with several experimental labs, both within and outside of the CCU.

- **Dr. Mainen Lab**

  <u>Optogenetic identification and control of serotonin neurons in behaving animals</u>
  Serotonin is an important neurotransmitter implicated in a wide variety of physiological functions and pathophysiologies but whose function is not well understood. Critically, very little is known about the activity of serotonin-releasing neurons in the brain. This problem is greatly exacerbated by the difficulty in their identification during physiological recordings. To address these problems we are using a combination of behavioral analysis, electrophysiological recording and optical-genetic probes targeted through specific promoters to this class of cells. By selectively activating serotonin neurons with light delivered through implanted fiber optics, we will be able to positively identify them during recordings and to specifically activate them, allowing us to test specific hypotheses concerning the role of serotonin in brain function and behavior.
  Funding: ERC

Olfactory objects and decisions: From psychophysics to neural computation

Object recognition is an important and difficult problem solved by the nervous system. Although visual recognition is far more familiar to us, it is through the chemical senses that object recognition occurs for most organisms. Neural computations within the olfactory system enable faithful recognition and tracking of meaningful odor sources, even when they comprise complex chemical blends embedded in a sea of background odors. The overall aim of this line of work is to understand the neural computations that make olfactory object recognition possible. According to theoretical accounts, object recognition can be understood as a process of probabilistic inference. Under this hypothesis, complex odor stimuli are represented using a probabilistic population code and processed in a Bayesian optimal fashion by the nervous system. To link these normative ideas to specific neurophysiological and behavioral predictions, we are formalizing them using computational models. Experimentally, our main goal is to monitor and perturb object representations in the functioning, computing brain. To this end, we deploy psychophysical tasks in rats which formalize complex real-world olfactory problems and also allow us to operationalize cognitive processes such as attention and memory. By combining such quantitative paradigms with large-scale neural ensemble recordings in the olfactory cortex, we can study how populations of neurons encode and process complex odor scenes, attempt to account for behavioral performance, and test the predictions of theoretical models. At the level of neural circuits and their physiology, we are particularly interested in the origin of neuronal variability, the nature of inter-neuronal correlations, the properties of inter-areal brain communication and the action of neuromodulators.

Colaborators: Alex Pouget (U. Rochester), Matthieu Luis (CRG, Barcelona)

Funding: HFSP

Evaluating the reliability of knowledge: Neural mechanisms of confidence estimation

Humans and other animals must often make decisions on the basis of imperfect evidence. What is the neural basis for such judgments? How does the brain compute confidence estimates about predictions, memories and judgments? Previously, we found that a population of neurons in the orbitofrontal cortex (OFC) tracks the confidence in decision outcomes. We are seeking to extend these observations by testing whether confidence-related neural activity in the OFC is causally related to confidence judgments. We are also addressing how the uncertainty about a stimulus in the course of decision-making is computed in olfactory sensory cortex. These experiments will give us further insights into the nature of the neural processes underlying confidence estimation.

Colaborators: Adam Kepecs (CSHL)

Frontal cortex and the control of impulsive action

Inhibition of behaviour is as important as its generation, and failure to inhibit inappropriate actions—impulsivity—is a central feature of pathologies including attention deficit hyperactivity disorder, drug addiction and obsessive compulsive disorder. Previous work has identified the frontal cortex as a central component in the control of inhibiting impulsive actions. The goal of this project is to understand how this brain area performs this function. Two current specific aims are to reveal the activity of frontal cortical neurons while rats are

engaged in impulse control task and to examine the effect of inactivating subregions of frontal cortex on impulse control behavior. Recording from large ensembles of neurons in the medial prefrontal cortex (mPFC) and the secondary motor cortex (M2) of rats during performance of the impulse control task allows us to characterize in detail the neural activity in these areas in relationship to behavior. We find that the activity of subpopulations of mPFC and M2 neurons predict the impulse control performance of rats on a trial-by-trial basis. Preliminary results show that reversible inactivation of the mPFC also impairs the ability of rats to inhibit impulsive action. We are now seeking to understand in more detail the nature of the neural representations underlying impulse control.

- **Dr. Moreno Lab**

  We are generally interested in how cells of multicellular animals can detect the fitness of neighboring cells selecting the fittest cells. This is a fundamental process that has implications in several broader fields, for example: 1. Ageing: During ageing, elimination of unfit cells maintains tissue health and prolongs lifespan (Merino et al., Cell 2015). 2. Cancer: Tumor cells can behave as superfit cells and expand by killing and replacing neighbouring tissue, leading to tissue invasion and destruction (Levayer et al., Nature 2015). 3. Neurobiology: Neurons can also exchange fitness information leading to the culling of less fit neurons during development (Merino et al.Curr. Biol., 2013), brain regeneration (Moreno et al., Curr. Biol., 2015) and brain ageing (Merino et al., Cell 2015).

- **Dr. Paton Lab**

  <u>Neural encoding of timing in the rat striatum</u>
  The striatum forms a major input structure for the basal ganglia, and is a site of degeneration in diseases such as Parkinson's and Huntington's disease. It receives input broadly from cortex and thalamus, as well as from neuromodulatory systems, and funnels the resulting activity into a successively decreasing number of neurons before transmitting its output to the thalamus. This architecture is thought the facilitate selection of representations of actions, events, thoughts and relationships between them, from its various input patterns. Interestingly, perturbing normal function there through lesions or pharmacology can cause deficits in timing behavior, and, depending on site and type of manipulation, different aspects of learning. By parametrically varying a time interval that a rat estimates, while simultaneously recording action potentials from single neurons in the striatum, we aim to identify neurons whose activity is correlated with a change in estimated interval. The signals we identify will provide a starting point for modeling efforts as well as perturbation studies, wherein specific neural signals can be manipulated. We have begun by recording from neurons in the striatum of the rat. We will test the hypothesis that some of these neurons will shift their response rate and/or latency as we shift the time interval that rats estimate. By identifying neurons with such "tuning" for a temporal interval, we hope to identify a neural substrate for the time-based computations that may underlie learning.

In the past year, we have succeeded in training rats to press a lever in order to gain rewards at defined intervals, classically called operant conditioning on a Fixed Interval (FI) reinforcement schedule. We have adapted the classical FI schedule such that in blocks of 30 trials, on average, we shift the fixed interval over a range of about one minute. We call this schedule a Serial Fixed Interval (SFI) schedule of reinforcement and have analyzed rats' behavior and initiated neurophysiological recordings during this task. Rats normally start responding just after the midpoint of the reinforcement interval, peaking around the time of reinforcement. We then shift the interval of reinforcement in blocks of trials to a different interval and animals shift the time at which they begin to respond, thus giving us a behavioural readout that reflects the animals changing knowledge about time until reward. Animals learn the interval associated with each block quickly, usually adapting their response times within five or fewer cycles of reward. This allows us to test animals on as many as ten distinct intervals that vary in duration over a range of about one minute during single sessions. This wide range of variation in estimated interval gives us statistical power when searching for neural correlates of timing behavior.

Optogenetic investigation of interval timing in mice

In the past year, we have initiated a parallel set of timing studies in mice in order to take advantage the increased molecular power of the mouse relative to the rat. We have trained mice on a classic temporal reproduction task, called the peak interval task, and are currently training mice on the SFI task mentioned above. By combining viruses dependent on CRE recombinase activity for expression of transgenes, with mouse lines expressing CRE in specific basal ganglia cell types, we plan to express light sensitive channels and pumps in targeted locations within the basal ganglia circuit. Stimulating these proteins with light during experiments will provide us with two potentially powerful pieces of data. First, we will be able to ask what type of cell we are recording from in vivo much more easily and in higher volume than was available with older techniques. Second, we can test hypotheses about the role of activity in specific populations of neurons for timing behavior.

- **Dr. Polavieja Lab**

We are developing new theoretical frameworks to understand collective behavior, including approaches adapting Bayesian Theory, Control Theory or Reinforcement Learning. We apply these approaches to better explain how decision-making, learning or group coordination takes place in groups.

Experiments in Zebrafish and Humans

Our setups include custom-made behavioral arenas for zebrafish, brain imaging in zebrafish, psychophysics and virtual reality for humans and molecular biology.

Machine Learning and Artificial Intelligence

We are developing and adapting supervised and unsupervised techniques from machine learning and artificial intelligence for extraction of relevant data in experiments and to quantify individual and collective behavior.

- **Dr. Rhiner Lab**

  We are interested in understanding the molecular mechanisms that drive and stop the activation, proliferation and differentiation of quiescent stem cells with implications for tissue homeostasis, cancer and regeneration.

  Stem cell control
  Many mammalian tissues contain pools of quiescent adult stem cells, which are only activated in pathological conditions or upon injury and have been proposed to act as a backup population. Regulation of stem cell quiescence and the intrinsic mechanisms by which cells sense and respond to injury-related signals are not well understood.
  To unravel such mechanisms, we study recently discovered quiescent adult neural stem cell in the genetically accessible model organism Drosophila, which are activated upon traumatic brain injury by stab lesion (Fernandez-Hernandez et al., 2013)
  To reach this goal, we perform whole genome expression profiling (microarrays and RNAseq) in combination with powerful functional RNAi assays and expression studies to validate candidate genes, followed by detailed characterization of conserved components.

  Regenerative neurogenesis and function
  We also seek to answer fundamental questions such as how adult-born neurons integrate into the mature brain and if their integration contributes to recovery of brain function after injury. To this end, we apply high-end microscopy, electrophysiology and behavioral assays to monitor performance after injury.

- **Dr. Ribeiro Lab**

  Molecular and neuronal mechanisms of nutrient choice
  How do animals know what type of nutrients they need? What are the molecular and neuronal mechanisms used by the brain to change the behavior of the animal to allow it to find and eat the required nutrients? We are analyzing genes identified as being required for nutrient choice in a neuronal whole-genome RNAi screen. Contemporaneously we have used genetic approaches to identify neuronal populations which are required for the same nutrient choices. The knowledge of molecular and neuronal players will be an entry point for studying neuronal mechanisms of nutrient balancing at the molecular, cellular and circuit levels.

  Quantitative analysis of feeding behavior in Drosophila
  Drosophila has become a powerful model organism in neuroscience research not only due to its molecular genetics toolkit, but also due to the successful development of methods and protocols to monitor and annotate behavior. Feeding and foraging are central elements in a majority of behavioral assays, but their quantification and analysis is a major challenge in the fly. We have developed flyPAD – fly Proboscis and Activity Detector, a method to automatically monitor feeding behavior quantitatively in individual flies. Our method is based on capacitive measurement of a fly's interaction with the food. The precision of the

measurements allows for high fidelity, high temporal resolution, and unbiased measurements of feeding behavior. We demonstrate that flies ingest food by rhythmically extending their proboscis with a frequency that is not modulated by the internal state of the animal. Instead, hunger and satiety homeostatically modulate the microstructure of feeding. These results highlight similarities of food intake regulation between insects, rodents, and humans, pointing to a common strategy in how the nervous systems of different animals control food intake. This method complements our continuing experimental and quantitative modeling approaches to understand how the internal state affects foraging and feeding strategies to achieve nutrient homeostasis.

- **Dr. Shemesh Lab**

Deciphering distributed neural circuits via advanced fMRI coupled with optogenetics

Complex behaviors ultimately arise from neural activity in widespread, distributed systems in the brain. We are interested in deciphering such networks in awake behaving rodents via optogenetics and advanced ultrafast fMRI. To achieve this goal, we are currently developing optogenetics- and MRI-compatible behavioral paradigms for awake rodents, as well as advanced ultrafast MRI acquisition strategies that will enable the resolution of relatively fast dynamics. The long term goals include the identification of distributed circuits and the investigation of their causal dynamics.

Functional MRI via non-BOLD mechanisms

The success of functional-MRI (fMRI) stems from its ability to portray active brain regions upon prescribing a specific task. However, fMRI relies on the Blood-Oxygenation-Level-Dependent (BOLD) mechanism, which is a surrogate marker for neural activity via neurovasculature couplings. A major goal of the Lab will therefore be to harness MRI's versatility – especially at the ultrahigh fields – towards capturing signatures for neural activity more directly. Specifically, we are interested in detecting cellular swellings upon activation, as well as neurotransmitter releases in the activated regions. Both phenomena can be considered epitomes of neural activity, and their direct detection is expected to provide much insight into the nature of the ensuing activity. We are investigating these phenomena – as well as BOLD neurophysiology – via MRI coupled to orthogonal modalities such as optical microscopy and optogenetics, in numerous settings from organotypic cultures (where hemodynamics are absent) to in vivo rodents.

Microstructural determinants of functional modulations leading to behavioral changes in healthy and diseased CNS

Modulations in brain function (e.g., enhancements arising from plasticity or aberrations arising from neurodegeneration) are intimately correlated with underlying micro-architectural modifications in the neural tissues. We are interested in studying the links between the two, in vivo, in a longitudinal fashion in animal models of plasticity on the one hand and neurodegeneration on the other hand. We investigate functional modulations (such as neural network reorganizations) using optogenetics as the specific source of stimulation, and BOLD- and nonBOLD-fMRI as the functional readouts. We augment this

functional information with advanced in vivo MRI methodologies that are selectively designed to probe even subtle changes in microstructures arising from plasticity or, conversely, neurodegenerative processes. We target microstructural changes in white matter, where we study variations in axonal size distributions (that govern the conduction velocity) as well as in gray matter, where we study changes in randomly oriented tissue components. We further aim to investigate the diagnostic potential arising from the identification of structural changes preceding functional/behavioral modifications.

- **Dr. Veiga-Fernandes Lab**

  Our long-term goal is to understand sensory and communication pathways that determine immune cell fate and disease progression. To achieve this, we centre our efforts at mucosal barriers that constitute the largest interface of the body with the external environment. Epithelial barriers line body surfaces and grant safeguard against harmful pathogens but are frequent targets of oncologic transformation and chronic inflammatory diseases. In this context, we tackle the following hitherto elusive points:

  Micronutrient sensing by lymphocytes

  We aim to unravel the mechanisms by which micronutrients control mucosal lymphocytes and their interaction with the epithelial barrier and microbiota.

  Multi-tissue sensors

  We focus on novel sensing programs by which lymphocytes integrate neuron and neuroglia cues, defining key multi-cellular units at the core of mucosal physiology in health and disease. Neuronal circuits and circadian inputs: we focus on how neuronal circuits and their inputs regulate immune functions in the context of chronic inflammation and cancer.

Qualifications: *IIP candidates with interests in neuroscience, health, medicine, and cancer research are encouraged to apply. Experience at a clinic or In a laboratory would be an asset.*