The ability to shape behavior based on the consequences of actions is fundamental for the survival of animals in complex environments. The neural mechanisms underlying this type of operant learning have been studied intensely in mammals, and are thought to be dysfunctional in a number of neurological and neuropsychiatric disorders in humans including addiction,
Parkinson’s disease, and autism. Prior studies have identified cortico-basal ganglia circuits as an important locus of operant learning function in the brain. The striatum, the major input structure of the basal ganglia (BG), receives inputs from a broad set of regions, including, in mammals, most of the cortex and thalamus, as well as subcortical areas, and a prominent feature of BG circuits is the existence of parallel loops in which the outputs are connected back to the areas from which their inputs originated. A comprehensive understanding of BG contributions to operant learning would benefit greatly, therefore, from the ability to observe simultaneously activity across a diverse set of brain areas during behavior. Additionally, knowledge of the identity of neural circuit elements beyond what is reflected in their anatomical location is essential for building an accurate circuit-level understanding of learning.

Zebrafish, a model organism with a substantial toolbox of genetic methods, is very well suited to such integrative approaches. At early life stages, they show a variety of robust innate and learned behaviors, while their brain, which has one million times fewer neurons than a human’s, and is less than a billionth of the size, already follows the basic vertebrate blueprint. Recent advances in optical and genetic technologies have made it possible to image activity, in real-time and with cellular resolution, non-invasively from throughout the entire brain. Since zebrafish are capable of operant learning, they can provide a powerful system to investigate its underlying circuit mechanisms.

In mammals, striatal projection neurons fall into two main types based on immunohistochemistry and anatomy. Direct pathway neurons express D1 dopamine (DA) receptors and substance P and project directly to BG output nuclei. Indirect pathway neurons express D2 DA receptors, enkephalin, and largely send information to BG output nuclei indirectly, with a sign inversion. It was recently shown in mice that activation of direct and indirect pathway neurons produces opposite effects on reinforcement. Stimulation of direct/indirect pathway neurons positively/aversively reinforced actions respectively. Teleost fish possess a similar divergent circuit architecture in the homologous structure to the mammalian striatum. We aim to interrogate these circuits using a combination of genetic methods, whole-brain calcium imaging and optogenetics to better understand their functional organization at a cellular level and to elucidate basic mechanisms of operant learning.

Atividades | Genetic dissection of striatal output pathways in zebrafish

- Development of novel learning assays in larval fish
  - Design and validation of assays for learned behavior in larval fish that are compatible with recordings of whole-brain physiology
- Mapping population activity dynamics during learning
  - Recording activity from the whole brain, and from genetically labeled populations, using genetically encoded sensors during learning
- Optical and genetic perturbations of neural activity
  - Perturbing specific neural populations using genetics, optical methods and pharmacology to assess the affect on learning.

Resultados atingidos e em progresso | Development of transgenic lines to label striatal pathways using recently developed CrispR
knock-in methods. Specifically we developed two lines to label the homolog of the ‘indirect pathway’ by knocking GAL4FF into the genomic locus upstream of the adora2aa and penkb. Development of lines to express the latest generation of optogenetic effectors.

Characterization of the anatomy of the larval pallium and the subpallium, the fish homolog of the striatum, using the lines developed above, as well as lines developed and shared by collaborators (Marnie Halpern, Dartmouth, USA) (work in preparation).

Development of a novel head-fixed learning assay for larval zebrafish, using a delay conditioning paradigm, and based on using the photochemical substrate optovin as an unconditioned stimulus (US). Further adaptation of this assay to develop a Trace Conditioning paradigm, a behaviour which has not been previously described for zebrafish larvae. This work has also been presented in several National and International Meetings. We expect to publish these novel conditioning assays soon in a paper, as well as in the PhD Thesis of the student Joaquim Contradanças.

Development of a modular, open source application for tracking behavior in head-fixed larval zebrafish, that enables the necessary software capability and hardware integration for these learning experiments. This software is written in C# using a framework developed in our lab, and allows for easy modular integration with hardware control systems, and behavioral protocols. The software package is available here (https://bitbucket.org/fchampalimaud/reference-modular-head-restrained-tracker/src/master/).

Developed of computational methods to analyze high-speed behavioral data and allow easy integration across different systems, assays and labs (MEGABOUTS). This work has been presented in international meetings and we are currently preparing a paper for publication, and an open source software package to be freely shared. These methods have also already been used in collaborations with other labs to study the effects of specific mutations on learning and locomotion, with 3 manuscripts currently in revision.

Use of the assays above in combination with whole-brain imaging to study the neural dynamics that accompany long timescale changes in behavioral state and the learning of conditioned responses, and particularly the role of the striatal pathways in setting the rate of locomotion. For this purpose, we built a SCAPE (swept confocally aligned planar excitation) microscope from a design shared by Elizabeth Hillman (Columbia University, NY, USA) which allows whole-brain scans at rates of 5-20 volumes per second.

Development of a new transgenic line giving pan-neuronal expression of a new variant of the neural activity integrator, CaMPARI, shared ahead of publication by Eric Schreiter (Janelia Farm Research Campus, USA).

Outputs:

Submitted preprints:


Posters and oral presentations including work and methods developed as part of this project:


Websites and software:

Software for integrating fish behavioral tracking and microscopy
https://bitbucket.org/fchampalimaud/reference-modular-head-restrained-tracker/src/master/HeadRestrainedTracking/

Other Activities:

Michael Orger co-organized Zenith European Training Network Course on Genetics and Imaging in Champalimaud Foundation, November 2021. Practical demonstrations of behavioral assays, software and imaging systems. November 2021