Designação do projeto| Mecanismos de condicionamento operante no cérebro inteiro da larva de peixe-zebra: células, circuitos e comportamento

Código do projeto|LISBOA-01-0145-FEDER-32664

PTDC/MED-NEU/32664/2017

Objetivo principal|

Região de intervenção | Lisboa

Entidade beneficiária| FUNDAÇÃO D. ANNA DE SOMMER CHAMPALIMAUD E DR. CARLOS MONTEZ CHAMPALIMAUD

Data de aprovação| 16/04/2018

Data de início| 17/07/2018

Data de conclusão|16/01/2022

Custo total elegível|228 559,10€

Apoio financeiro da União Europeia| FEDER: 91 423,64€

Apoio financeiro público nacional/regional|137 135,46€

Objetivo|

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 lines to target genetic tools to neural populations in the zebrafish forebrain using the GAL4/UAS system

1) 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
2) Mapping population activity dynamics during learning
   Recording activity from the whole brain, and from genetically labeled populations, using genetically encoded sensors during learning
3) 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:

Mouret RZ, Greenbaum JP, Doll HM, Brody EM, Iacobucci EL, Roland NC, Simamora RC, Ruiz I, Seymour R, Ludwick L, Groneberg AH, Marques JC, Laborde A, Rajan G, Del Bene F, Orger MB, Jain RA (2022). The Adaptor Protein 2 (AP2) complex modulates habituation and behavioral selection across multiple pathways and time windows. bioRxiv 2022.05.20.492863 (Currently in revision at iScience)

Felix R, Markov DA, Renninger SL, Tomás R, Laborde A, Carey MR, Orger MB* and Portugues R* (2021). Structural and functional organization of visual responses in the inferior olive of larval zebrafish. bioRxiv 2021.11.29.470378 (Currently in revision at Journal of Neuroscience)

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

2019 Poster. Deep Learning Toolbox for classification and analysis of zebrafish larvae behaviour. Alexandre Laborde, Adrien Jouary and Michael Orger. Edin Fish Tech August 2019. Edinburgh, UK.

2021 Oral presentation. Boehringer Ingelheim North America Meeting (held online due to pandemic). A novel head-fixed conditioning paradigm for larval zebrafish. Joaquim Contradan§as, Raquel Jacinto, Edite Figueiras, Alexandre Laborde, Joe Paton, Michael Orger.

2019 Oral presentation in "Quantifying Behavior as a Lens into the Brain." Modeling the Dynamics of Visual Behaviors in Zebrafish. Michael Orger. Society for Neuroscience Meeting, Chicago, USA.

2019 Poster presentation in "Quantifying Behavior as a Lens into the Brain." Modeling Behavioral Dynamics in Zebrafish. Adrien Jouary, Christian Machens and Michael Orger. Society for Neuroscience Meeting, Chicago, USA.

2021 Poster presentation. A novel head-fixed conditioning paradigm for larval zebrafish. Joaquim Contradangas, Raquel Jacinto, Edite Figueiras, Alexandre Laborde, Joe Paton, Michael Orger. Champalimaud Research Symposium, Lisbon, October, 2021.

2019 Poster presentation. Developmental characterization of neuronal subpopulations involved in visually guided behaviors. Bernardo Esteves, Joao Martins, Mariana Viegas, Sabine Renninger, Michael Orger, Ruth Diez del Corral. 3rd AXON Meeting, Alicante (Spain)

2018 Poster presentation. Developmental characterization of neuronal populations involved in visually guided behaviors Joao Martins, Bernardo Esteves, Sabine Renninger, Michael Orger, Ruth Diez del Corral. Meeting of the Portuguese Society for Developmental Biology, Porto (Portugal).

2019 Invited oral presentation. Functional dissection of visuomotor circuits in the zebrafish brain. Michael Orger. Spanish and Portuguese Advanced Optical Microscopy 2019

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