Data Management Plan

Project: RoCSMAP (ANR-23-CE16-0017)

Coordinator : Elim HONG (Neuroscience Paris Seine – IBPS – Sorbonne Université)

Model: ANR – DMP Template (English)

Summary

Proper response to external stimuli is essential for survival. Upon aversive stimuli animals display a stereotypical sequence of motor responses consisting of flight and/or freeze followed by recovery to baseline activity. While these motor responses cannot be executed simultaneously, they do however, occur sequentially. Aberrant response to aversive stimuli leads to depression, anxiety or addiction in humans. While studies have identified neuronal circuits that mediate aversive behavior in distinct brain regions, the biological correlates for how one circuit is selected over the other, a symmetry-breaking step known as competitive selection, is yet unclear.

The evolutionarily conserved habenulo-interpeduncular nucleus (Hb-IPN) pathway has emerged as a crucial brain region that mediates fear and stress-related behaviors. This pathway is composed of two distinct circuits, the cholinergic and the peptidergic non-cholinergic, that innervate adjacent domains in the IPN. We recently found a hardwired mode of negatively correlated activity between the cholinergic and non-cholinergic circuits, whereby the synchronized activation of cholinergic neurons inhibits non-cholinergic neuron activity. This occurs through retrograde GABAB signaling at the IPN via GABAB receptors located on the non-cholinergic terminals. An aversive stimulus, electric shock also induces this mode of negatively correlated activity. We hypothesize that different external stimuli induce competitive selection in the habenulo-interpeduncular nucleus pathway to modulate the stereotypical motor response. By taking advantage of the small size and transparency of the zebrafish larval brain to easily monitor and modify neuronal activity in head-fixed animals during behavior, this project aims to understand how an evolutionarily conserved asymmetric structure in the brain that integrates various external stimuli utilizes an atypical mode of competition to modulate motor responses to aversive stimuli.

1. DATA DESCRIPTION AND COLLECTION OR RE-USE OF EXISTING DATA

Work package 1: Elucidate how sensory stimulations affect distinct motor sequences by recording locomotor trajectories during behavioral assays.

1a. How will new data be collected or produced and/or how will existing data be re-used?

Experiment: To have a clear understanding of motor behavior during and following aversive stimuli (light, somatosensory stimuli), the Hong lab will record the locomotor response in both freely moving and head-fixed 6 day old larvae using Zebrabox (ViewPoint) and custom-built setups. The first set of experiments will be carried out in WT larvae. The second set of experiments will be performed in larvae with attenuation in neurotransmission in the habenulo-interpeduncular nucleus (Hb-IPN) pathway using genetic manipulation (mutants). We will also carry out the experiments on two different mutants that have reduced the excitatory vs. inhibitory neurotransmission in this part of the brain.

Data provenance will be indicated when the behavioral data is uploaded onto a public repository following the publication of the scientific manuscript, as well as in the materials and methods section of the paper.

There are no existing data sources that we are aware of outside of the preliminary data generated in our lab.

1b. What data (for example the kind, formats, and volumes), will be collected or produced?

A 30 min video recording of zebrafish locomotion at 25Hz will be acquired as a 525Mb mpeg file. We expect to have around 200 recordings consisting of 4 different conditions with 3 different intensities resulting in around 100 GB of data for WT larvae. Since we will repeat the experiments using 2 mutant larvae, we expect a total of 300 GB of data for the behavioral recordings.

Work package 2: Elucidate how sensory stimulations recruit neurons in the habenulo-interpeduncular nucleus (Hb-IPN) pathway by volumetric calcium imaging

1a. How will new data be collected or produced and/or how will existing data be re-used?

Experiment: The Hong lab will carry out simultaneous recording of the motor response and Hb-IPN pathway activity by volumetric calcium imaging using 2-photon light sheet microscopy in head-fixed 6 day old larvae expressing GCaMP only in the Hb-IPN pathway [*Tg(gng8:Gal4;UAS:GCaMP7*)]. We will use the stimulation protocol described for the behavioral recordings above. We will record the activity of habenular soma as well as in the axon terminals at the IPN. This experiment will be carried out in WT larvae as well as in the two mutant backgrounds described in the section above. In addition, the Wyart lab will carry out the same experiment in their custom built microscope to combine optogenetic stimulation of specific habenular neuronal populations with calcium imaging and behavioral recording (tail movement in agarose head-fixed larvae). The optogenetic stimulation will be performed to either excite (ChrimsonR-tdTomato) or inhibit (eNpHR3.0-eYFP) specific neuronal populations in the Hb-IPN pathway.

Data provenance will be indicated when the calcium recording data is uploaded onto a public repository following the publication of the scientific manuscript, as well as in the materials and methods section of the paper.

There are no existing data sources that we are aware of outside of the preliminary data generated in our lab.

1b. What data (for example the kind, formats, and volumes), will be collected or produced?

A 30 min time-lapse recording of 13 z-focal layers at 4Hz speed/volume of calcium imaging results in a 10G tif file. We expect to have around 10 recordings per genotype (WT, *vachtb, gabbr1a* mutants) as well as for the two different optogenetic stimulations (20 larvae per stimulation) consisting of single and combined external stimuli presentations with a total of 210 recordings ((10x3 (gene) + 20x2 (opto))x3 (stimuli)). We anticipate around 2.1 TB (210 recordings x 10G) of calcium imaging data.

Work package 3: Identify the functional downstream targets of the Hb-IPN pathway.

1a. How will new data be collected or produced and/or how will existing data be re-used?

Experiment: The Hong and Wyart labs will use high-speed volumetric calcium imaging to identify how the Hb-IPN pathway recruits downstream targets in larvae that co-express GCaMP in all neurons [*Tg(elav3-GCaMP6m*)] and opsins only in the Hb-IPN pathway. The calcium imaging experiments combined with optogenetics and behavioral recording (above) will have informed which neuronal populations in the Hb-IPN pathway has behavioral consequences. This information will be used to target already-identified neurons in the Hb to map its downstream targets in the brain.

1b. What data (for example the kind, formats, and volumes), will be collected or produced?

A 5 min time-lapse recording of 60 z-focal layers at 4Hz speed/volume of calcium imaging results in approximately 10Gb tif file. We anticipate a total of 40 recordings per optogenetic stimulations (20 recordings x 2 stimulations) resulting in a total of 400 Gb data. The calcium imaging will be combined with behavioral monitoring at 40Hz leading to 100Gb per file and 4Tb of data to be tracked under 600Gb and kept in the compressed tracked format on the Paris Brain Institute NAS server. Total of data raw and preprocessed: 5Tb.

2. DOCUMENTATION AND DATA QUALITY

1a. What metadata and documentation (for example way of organising data) will accompany the data?

All data will be generated in OME-XML format. The name of each experiment will have a unique ID. A 'readme' text file for each set of experiments will be made available. A detailed description of the date, identifier, age, genotype, stimulus, conditions, microscope and experimenter will be included in the text tile. A metadata file following international standards (i.e. Datacite) will be included with the dataset
The metadata information as well as a 'readme' text will also be available in a physical lab notebook.

2b. What data quality control measures will be used?

For calcium imaging and behavioral recordings, data quality control will be insured by reproducibility of biological output and peer review of data within and outside the consortium.

For calcium imaging, signal to noise ratio in the calcium transients of the habenular pathway will reach at least 4 to proceed to an extensive analysis.

For behavioral analysis, tracking of the tail position will be implemented using ZebraZoom and manually checked on hundreds of bouts.

A physical lab notebook that details the quality of the data is logged for each experiment and kept in the office of the lab members.

3. STORAGE AND BACKUP DURING THE RESEARCH PROCESS

3a. How will data and metadata be stored and backed up during the research?

All data will be stored in an external hard drive in addition to two additional secured servers in the Sorbonne Université. The servers are provided by the department as well as at the home research institute (ArtBIO, Sorbonne University, France), which are fully secured by a team of IT support service. The server in the home research institute is located in a different building from the lab and department server room. All external server rooms are secured and requires ID pass/key for access. Backup is automated each week in addition to personal backup on hardrive.

Long-term storage: external hard drive 15 years after publication

3b. How will data security and protection of sensitive data be taken care during the research

Each home research institute server is equipped with its own data safety storage and backup plan. As there are no human-related data in this project, there will be no high security sensitive data being produced.

4. LEGAL AND ETHICAL REQUIREMENTS, CODE OF CONDUCT

4a. If personal data are processed, how will compliance with legislation on personal data and on security be ensured?

The experiments are carried out in zebrafish larvae. There will be no personal data produced.

4b. How will other legal issues, such as intellectual property rights and ownership, be managed? What legislation is applicable?

All data generated will be available to the consortium: experimentalists involved in data acquisition, PI involved in strategy, data analysis, equipment setup and securing funding will be systematically involved in the IP. The precise intellectual property rights, degree of collaborative implication and ownership will be discussed on a regular basis during the generation of data and agreed upon when the manuscript or other research output are published.

4c. What ethical issues and codes of conduct are there, and how will they be taken into account?

The experiments have been designed to follow The European Code of Conduct for Research Integrity (2023). https://allea.org/wp-content/uploads/2023/06/European-Code-of-Conduct-Revised-Edition-2023.pdf
The animal welfare ethical committee have reviewed and authorized the animals and/or protocols described in the ANR - RoCSMAP project. Any significant deviation/modification of the animals and/or protocols will be submitted to the committee for further approval.

5. DATA SHARING AND LONG-TERM PRESERVATION

5a. How and when will data be shared? Are there possible restrictions to data sharing or embargo reasons?

The data will be shared at any moment during the project within the consortium.
The data will also be available to be presented during any communications during congress prior to publication upon agreement within the consortium.
Once the manuscript is published, data will be accessible upon request to the authors as well as placed in the archives service of the Sorbonne University. The data will be retained within the lab up to 10 years following publication and then archived at the Sorbonne University Archives. A free license will be associated with the dataset in order to specify the conditions of reuse and that the information for citation will be associated in the metadata file.

5b. How will data for preservation be selected, and where data will be preserved long-term (for example a data repository or archive)?

A long-term data preservation strategy will be defined with the archives service of Sorbonne University.

5c. What methods or software tools are needed to access and use data?

The behavioral recordings can be opened using any video player. The calcium imaging recordings are in a tif file, and can be opened using FIJI, an open access software for image processing.

5d. How will the application of a unique and persistent identifier (such as a Digital Object Identifier (DOI)) to each data set be ensured?

DOI will be assigned through the online archiving process.

6. DATA MANAGEMENT RESPONSIBILITIES AND RESOURCES

6a. Who (for example role, position, and institution) will be responsible for data management (i.e. the data steward)?

All partners in the consortium and every person who generates data will comply with the backup policy and archiving aforementioned. The responsibility of the data management will be on the PI in which the data is generated from. Scientists directly involved in the project management share co-ownership of the DMP and on a regular basis will participate to the implementation and update of the DMP.

6b. What resources (for example financial and time) will be dedicated to data management and ensuring that data will be FAIR (Findable, Accessible, Interoperable, Re-usable)?

Only the cost of external hard drives were factored into the project cost whereas, data curation time and human resource were not included. Data management will be carried out in accordance with the principles of FAIR during the data life cycle.