**DATA**

Definition of terms

master list – Google spreadsheet containing details of all mice data and the completed processing steps

session – an imaging session, usually means a

environment – circular track for Neurotar system

e.g. fam1, fam2 ‘fam’ denotes a familiar environment, one where a mouse has been trained in for

>6 sessions

nov ‘nov’ denotes a novel environment, one which the mouse encounters for the first

time during imaging

fam1rev ‘rev’ denotes reverse direction

experiment – imaging experiment with animal in a single environment or moving between environments. An

animal typically only has one experiment per day.

e.g. fam1 animal imaged while in fam1

fam1fam1rev animal imaged first in fam1, then in fam1rev

fam1fam2fam1 animal imaged first in fam1, then in fam2, then back in fam1

run – an occurrence of an environment within an experiment

e.g. In the experiment fam1fam2fam1, there are 3 runs: fam1, fam2 and fam1r2. For clarity, these runs

are named

fam1fam2fam1-fam1

fam1fam2fam1-fam2

fam1fam2fam1-fam1r2

recording – an image file that’s usually 4 min long

**Existing mice data**

All mice data and the details of their experiments and processing are catalogued in this [master list](https://docs.google.com/spreadsheets/d/1CV_TNpRU9opL2GwOkhLNzVcO9koPhHPp/edit#gid=1521373079). Each tab of the spreadsheet corresponds to an animal and data for each animal is organised first by FOV (for cases of multiple fovs – fields of view), then by experiment. Lists for the different experiments which are used in the processing scripts can be found in

thefarm2/live/CrazyEights/AD\_2PCa/Digital\_Logbook/lists\_imaging/

**Experiments**

Ideally, each mouse should have data for all imaging experiments below:

fam1fam2fam1

fam1novfam1

fam1fam1revfam1

Some experiments are short and do not have the return to fam1. For example,

fam1fam2

fam1nov

fam1fam1rev

Also, for different reasons (headplate could come off, mouse could die, etc.), a mouse may only have a subset of the above experiments. Mice are imaged for about ~30 min but the images are saved in 4-minute recordings (i.e. files).

**Raw data**

All experiment data are in

thefarm2/live/CrazyEights/AD\_2PCa/Data/

and are organised into folders named by day of experiment (yyyymmdd).

In each folder, there may be one or more of the following folders:

Neurotar – contains raw behaviour data

2P – contains raw two-photon calcium imaging data

Processed – contains processed data

Timestamps – contains timestamps for reward and for TTL triggers for imaging sent from Neurotar. Earlier

experiments do not have this folder

* Neurotar
  + Contains folders named either

Track\_yyyy-mm-dd-HH-MM-SS or

SavedTrack\_yyyy-mm-dd-HH-MM-SS

each of which has either a .csv (prior to 20181123) or .tdms (20181123 and later) file with the behaviour tracking data.

* + Tracking data is recorded at ~100 Hz and contains data on time, position (x, y, r, phi), speed, head direction, etc.
  + If the tracking file has been processed, there may be a .mat file in the folder also.
  + If no .csv or .tdms file exists, Neurotar failed to save the file. In the folder, there will be a .trset file (Neurotar’s temporary saving file) which should contain most of the tracking data. This file can be opened in the Matlab script window and saved as a tdms file. Then it can be processed as a regular .tdms tracking file.
* 2P
  + Contains folders named yyyymmdd\_HH\_MM\_SS\_2P each of which contains an image file

yyyymmdd\_HH\_MM\_DD\_2P\_XYT.raw or

yyyymmdd\_HH\_MM\_DD\_2P\_XYZT.raw (rare)

This file typically consists of a 4-minute recording in two channels (red and green, interleaved) at a frame rate of ~30 Hz (30.9) – a total of 14840 frames (7420 frames per channel).

* + If the image file has been processed, the folder should also contain the files

yyyymmdd\_HH\_MM\_DD\_2P\_XYT\_green.tif

yyyymmdd\_HH\_MM\_DD\_2P\_XYT\_red.tif

Each of these files will have 7420 frames.

**DATA PROCESSING**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Step | Relevant scripts in neuroSEE | Description | Output | User intervention |
| 1 Data quality check | 1 pipelines/frun\_pipeline\_batch.m  (dostep = [1;1;0;0;0;0]) | Batch processing (by list) of **motion correction** and **ROI segmentation** via CaImAn. If there are many experiments to be processed, instead of processing one list at a time, create one very long list of all the files to be processed so you only have to submit one job to HPC. | Motion-corrected images and ROI segmentation results for each file  For the file yyyymmdd\_HH\_MM\_SS, the motion-corrected files are saved as  thefarm2/…/AD\_2PCa/Data/yyyymmdd/Processed/yyyymmdd\_HH\_MM\_SS/mcorr\_normcorre/yyyymmdd\_HH\_MM\_SS\_XYT\_green\_mcorr.tif  thefarm2/…/ yyyymmdd\_HH\_MM\_SS\_XYT\_red\_mcorr.tif  And the CaImAn output is saved in  thefarm2/…/AD\_2PCa/Data/yyyymmdd/Processed/yyyymmdd\_HH\_MM\_SS/mcorr\_normcorre/CaImAn/  \*Note: Current motion correction is done with normcore (first with rigid correction, then non-rigid correction). But in the past, I did non-rigid correction only so the folder mcorr\_normcorre-nr might exist for some of the earlier files. |  |
| 2 quality\_control/frun\_getFileNrois.m | Retrieves number of ROIs for each file |  | Exclude files with negligible number of ROIs |
| 3 quality\_control/frun\_getFileCircLaps.m | Calculates number of laps for each recording |  | Exclude files with negligible number of laps |
| 4 quality\_control/frun\_collate\_indivproc\_results.m | Compares motion-corrected files, number of ROIs and laps for each file to help user choose the best reference file | Summary figures comparing motion corrected files, ROIs and animal activity for all files in a list. These are in  thefarm2/…/AD\_2PCa/Analysis/mXX/mXX\_fam1fam2-fam1/individual\_ proc/indiv\_normcorre\_CaImAn\_FISSA/ | Choose reference file for image registration |
| 2 Image registration | 1 pipelines/ frun\_mcorr\_batch.m | Batch processing (by list) of image registration to a reference file | Individual files registered to reference file  For the file yyyymmdd\_HH\_MM\_SS, the registered images are saved as  thefarm2/…/AD\_2PCa/Data/yyyymmdd/Processed/yyyymmdd\_HH\_MM\_SS/imreg\_normcorre\_refyyyymmdd\_HH\_MM\_SS/yyyymmdd\_HH\_MM\_SS\_XYT\_green\_imreg\_refyyyymmdd\_HH\_MM\_SS.tif  thefarm2/…/yyyymmdd\_HH\_MM\_SS\_XYT\_red\_imreg\_refyyyymmdd\_ HH\_MM\_SS.tif |  |
| 2 quality\_control/frun\_collate\_imreg\_results.m | Compares registered images | Summary figures comparing registered images. These are in  thefarm2/…/AD\_2PCa/Analysis/mXX/mXX\_fam1fam2-fam1/individual \_proc/imreg\_normcorre/ | Check that images were registered satisfactorily. This is important. If the image registration results are not acceptable, repeat this step with a different reference image or with a higher max\_dev value. |
| 3 ROI segmentation | 1 pipelines/frun\_pipeline\_imreg.m  (dostep = [1;1;0;0;0;0])  \* ROI elimination criteria parameters may be redefined in  neurosee/utilities/neurosee\_setparams.m | All image files in a list are concatenated and segmented for ROIs. List must be for experiment instead of individual run  **✓** ‘list\_...\_fam1fam2fam1.txt’  ✘ ‘list\_...\_fam1fam2fam1-fam1.txt’  ROIs are eliminated according to the following criteria:  1 ROIs touching image border (to within 4 pixels)  2 50<area<400 (490x490 fov)  70<area<560 (330x330 fov)  3 inverse circularity > 4  4 >25% area overlap in any 2 ROIs | ROIs for list  \*CaImAn also outputs the calcium timeseries. These are saved but we do not use them.  For the list, list\_mXX\_fam1fam2fam1.txt, the CaImAn output is saved in  thefarm2/…/AD\_2PCa/Analysis/mXX/mXX\_fam1fam2-fam1/group\_ proc/imreg\_normcorre\_CaImAn/mXX\_fam1fam2fam1\_imreg\_ refyyyymmdd\_HH\_MM\_SS/  Copies of the CaImAn output are also saved in the folders corresponding to each run  thefarm2/…/mXX\_fam1fam2fam1-fam1\_imreg\_refyyyymmdd/  thefarm2/…/mXX\_fam1fam2fam1-fam2\_imreg\_refyyyymmdd/  thefarm2/…/mXX\_fam1fam2fam1-fam1r2\_imreg\_refyyyymmdd/ |  |
| 4 Fissa correction (neuropil decontamination) | 1 pipelines/frun\_pipeline\_imreg.m  (dostep = [1;1;1;0;0;0]) | All image files in a list are concatenated and neuropil-corrected. List can either be for an individual run or an experiment | Raw and neuropil-decontaminated calcium timeseries  For the list, list\_mXX\_fam1fam2fam1.txt, the FISSA output is saved in  thefarm2/…/AD\_2PCa/Analysis/mXX/mXX\_fam1fam2-fam1/group\_ proc/imreg\_normcorre\_CaImAn/mXX\_fam1fam2-fam1\_imreg\_ refyyyymmdd\_HH\_MM\_SS/FISSA/  Copies of the output are also saved in the folders corresponding to each run  thefarm2/…/mXX\_fam1fam2fam1-fam1\_imreg\_refyyyymmdd/FISSA/  thefarm2/…/mXX\_fam1fam2fam1-fam2\_imreg\_refyyyymmdd/ FISSA/  thefarm2/…/mXX\_fam1fam2fam1-fam1r2\_imreg\_refyyyymmdd/ FISSA/ |  |
| 2 quality\_control/GUI\_manuallydeleteROIs.m | GUI for manual elimination of ROIs |  | Check for ROIs with weird timeseries or visibly not in CA1 |
| 5 Spike extraction | 1 pipelines/frun\_pipeline\_imreg.m  (dostep = [1;1;1;1;0;0]) | List must be for an individual run, not an experiment  Spike extraction requires subtraction of a baseline (default is 85th percentile of fissa-corrected dF/F trace). Too low a baseline means a lot of false negative spikes. Too high a baseline means too few spikes are extracted. I try several different baselines typically ranging from 80-90%. | Inferred spike trains  For the list, list\_mXX\_fam1fam2-fam1.txt, the extracted spikes are saved in  …/AD\_2PCa/Analysis/mXX/mXX\_fam1fam2-fam1/group\_proc/…  imreg\_normcorre\_CaImAn/mXX\_fam1fam2-fam1\_imreg\_...  refyyyymmdd\_HH\_MM\_SS/FISSA/bl\_prctile##/ |  |
| 2 quality\_control/manually\_refine\_spikes/ (needs checking) | GUI for tweaking parameters for spike extraction |  | (Optional check) |

**DATA ANALYSIS (in the works)**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Type | Relevant scripts in neuroSEE | Description | Output | User intervention |
| 1 Place field mapping | pipelines/frun\_pipeline\_imreg.m |  | For the run  mXX\_fam1fam2-fam1  pf mapping output is saved in  …/AD\_2PCa/Analysis/mXX/mXX\_fam1fam2-fam1/group\_proc/… imreg\_normcorre\_CaImAn/mXX\_fam1fam2-fam1\_imreg\_refyyyymmdd\_HH\_MM\_SS/FISSA/bl\_prctileNN/  All plots summarising pf mapping output are in  …/AD\_2PCa/Analysis/mXX/mXX\_fam1fam2-fam1/group\_proc/… imreg\_normcorre\_CaImAn/mXX\_fam1fam2-fam1\_imreg\_refyyyymmdd\_HH\_MM\_SS/… FISSA/bl\_prctileNN/PFdata/hist\_SI\_bitspersec/ |  |
| 2 Remapping | pipelines/run\_showRemapping\_2env.m  pipelines/frun\_showRemapping\_2env\_multiAnimals.m |  | ROIs are tracked across the two environments in an experiment and place fields are compared.  For the experiment  mXX\_fam1fam2  remapping data are saved in  …/AD\_2PCa/Analysis/mXX/mXX\_fam1fam2/remapping/imreg\_normcorre\_CaImAn\_FISSA/ mXX\_fam1fam2\_imreg\_ refyyyymmdd\_HH\_MM\_SS1 - yyyymmdd\_HH\_MM\_SS2  \*refyyyymmdd\_HH\_MM\_SS1 is the reference file for mXX\_fam1fam2-fam1  refyyyymmdd\_HH\_MM\_SS2 is the reference file for mXX\_fam1fam2-fam2 |  |