Tutorial

This is a brief tutorial to access the *in vivo* imaging data from the paper: Bocchio M*, Gouny C*, Angulo-Garcia D, Toulat T, Quiroli E, Baude A, Cossart R. *Hippocampal hub neurons maintain distinct connectivity throughout their lifetime*. Nature Communications (accepted for publication). Pre-print on BioRxiv: <u>https://doi.org/10.1101/813923</u>

The NWB format

Each recording is saved in a Neurodata Without Borders (NWB) file containing calcium imaging movie, detected ROIs, filtered DF/F traces, inferred spikes and locomotion epochs.

For more information on the NWB format: <u>https://www.nwb.org/how-to-use/</u>

The NWB file can be opened using APIs for MATLAB (<u>MatNWB</u>) and Python (<u>PyNWB</u>). It can also be accessed using <u>HDF5 viewer</u>.

Details on the NWB schema can be found here: <u>https://nwb-schema.readthedocs.io/en/stable/</u>

Organisation of the file

The file was compiled in MATLAB using the MatNWB API (NWB version 2.2.2) following the Calcium Imaging tutorial available <u>here</u>, with some modifications.

Metadata

Metadata with the details on the imaging experiment are included in the file (e.g. details on the mouse, virus used, experimenter, etc.). Details on the mouse can be found in the general_subject group.

Raw data

The calcium imaging movie (motion-corrected using <u>NORMCorre</u>) can be found in: acquisition \rightarrow TwoPhotonSeries

Processed data

ROIs (detected using <u>CalmAn</u>) are stored as masks in: processing \rightarrow ophys \rightarrow nwbdatainterface \rightarrow ImageSegmentation \rightarrow planeSegmentation

Filtered DF/F traces can be found in:

```
processing \rightarrow ophys \rightarrow nwbdatainterface \rightarrow Fluorescence \rightarrow roiresponseseries \rightarrow RoiResponseSeries
```

Inferred spikes (deconvolution using CalmAn constrained FOOPSI algorithm) are stored in: analysis \rightarrow deconvolution

Locomotion epochs are as a logical array in:

processing \rightarrow behavior \rightarrow nwbdatainterface \rightarrow TimeSeries

Here is an example code to load the data from one calcium movie in MATLAB using MatNWB:

```
filename = 'Dlx1-2-GFP_jRGECO1a_05_20200211.nwb';
read nwb = nwbRead(filename);
% load raw movie
movie = read nwb.acquisition.get('TwoPhotonSeries').data.load;
% load image masks (ROIs)
rois = read nwb.processing.get('ophys'). ...
   nwbdatainterface.get('ImageSegmentation'). ...
    planesegmentation.get('PlaneSegmentation'). ...
   image_mask.data.load;
% load df/f traces
df f traces =
read nwb.processing.get('ophys').nwbdatainterface.get('Fluorescence')...
    .roiresponseseries.get('RoiResponseSeries').data.load;
% load spike count array (from deconvolution)
spikenums =
read nwb.analysis.get('deconvolution').nwbdatainterface.get('TimeSeries').data.lo
ad;
% load locomotion epochs (treadmill sensor)
mvmEpochsLogic =
read nwb.processing.get('behavior').nwbdatainterface.get('TimeSeries').data.load;
mvmEpochsIndex = find (mvmEpochsLogic == 1);
restEpochsLogic = ~mvmEpochsLogic;
restEpochsIndex = find (restEpochsLogic == 1);
```

Analyses included in the paper can be reproduced using the following MATLAB code available on GitLab.

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