This is an introduction for codes to analyze axo-axonic synaptic plasticity on AISs by ChC (*Jung et al.,* *An adaptive behavioral control motif mediated by cortical axo-axonic inhibition,* 2023).

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1. MASTER.m

An example to set input parameters and execute codes sequentially. Recommend to run codes in this order – other cases are not tested. Input the following parameters:

pathlist\_FW % list of paths for folders containing each slice image set: control group [string in cell].

pathlist\_WR % list of paths for folders containing each slice image set: experimental group [string in cell].

% The results will be saved in the same folders of each image set

L1line\_FW % Lines for layer 1/2 boundary of each slice: control group [double in cell]

L1line\_WR % Lines for layer 1/2 boundary of each slice: experimental group [double in cell]

% Each line information is two columns of coordinates. For example, if you get the line ROI on ImageJ, you can load it using *ReadImageJROI* (in this package) and get its mnCoordinates.

mouseidFW % a vector containing the ID of mouse for each slice image set : control group

mouseidWR % a vector containing the ID of mouse for each slice image set : experimental group

zi % first sliver of the image stack (double) to analyze – should be same for all the slice images

zf % last first sliver of the image stack (double) to analyze – should be same for all the slice images

pixelL % pixel size in length (micron) - same for all the slice images

img\_Gephyrin% Name of image file for Gephyrin Channel (string) - same for all the slice images

img\_ChC % Name of image file for ChC Channel (string) - same for all the slice images

img\_AnkG % Name of image file for AnkG Channel (string) - same for all the slice images

1. RunMultipleDetection.m

Execute detection, filter AISs by position, draw SSE map, and save the results for each image stack in separate folders. Make ‘RESallFW’ and ‘RESallWR’ which contain all the measurements in a matrix.

1. AISDetection.m

Execute detection for each image stack in separate folders. Outputs saved in each folder are:

'Results\_Detection.mat' – results of measurements

'Results\_Analysis.mat' – results of measurements

'DetectionMap\_AIS.tif' – image of detected AISs

'DetectionMap\_ChC.tif' – image of detected ChC projections

To generate analysis and figures, use the following functions. You can use ‘RESs.mat’ to test and reproduct figures of the paper.

SSEmap\_group.m: Make SSEmaps (Fig8 e, f, g, h).

GetRES\_byMouse.m: Organize results by mouse (following input *mouseid*) (Fig8 m, n)

Prob\_byMouse: Show SSE probability distribution by mouse (Ext.Data.Fig8 b-e)

CDF\_byMouse.m: Make CDF plot (Fig8 k, l)

DfromL1vsSSE.m: Make plot for the position from L1/L2 layer (following input L1line\_FW or L1line\_WR) (Fig8j, Ext.Data.Fig7f)

Bootstr\_BinDifference.m: Run bootstrapping and confirm bin difference (Fig8 o, p, Ext.Data.Fig8f-i)

Bootstr\_FoldChange: Run bootstrapping and confirm change in each bin by ratio (Fig8 q, r)

Other files are for functions used.

mergeLabelM.m

RenumberLM.m

pointSourceDetection.m

measureLAIS.m

measurePreSSE.m

ReadImageJROI.m

selectAIS.m

loadtiff.m