**Guide for spike analysis**

**Data analysis pipeline**

Copy data

1. Copy the data folder from the recording computer to the server.

Preprocess data for Klusta

1. In load\_command\_A1.m, change the variables experimentName and sessionName. Run the first section
2. In the 2nd section insert the new parameters and run the section
3. In the 3rd section, modify selCh if necessary and run the section
4. Decide based on std and max which trials to keep
5. In the 4th section modify subTrialsForAnalysis accordingly. Run the section
6. In the 5th section modify gain (choose between 10 or 100, so that there are not too many data points exceeding 32768) and run the section
7. Run the 6th section, 7th and 8th sections
8. Copy the .prm file from another similar experiment folder to the „klusta analysis“ folder and adjust the .prm file name, the „experiment\_name“ (→ .dat file name) and „voltage\_gain“ variables accordingly in the .prm file
9. Clear variables (clearvars -except experimentName sessionName) and close all figures to empty memory

Run klusta analysis

1. Open the Anaconda Prompt (windows) or Terminal (Linux)
2. Type „activate klusta“ (Windows) or „source activate klusta“ (Linux)
3. Change directory to the klusta folder
4. Type „klusta“ + .prm filename. The klusta will run for about 10-30 min
5. Open the resulted file with „phy kwik-gui“ + <filename>.kwik
6. Open the SpikeDataLoading\_openEphys\_A1.m script in Matlab and with the experimentName and sessionName from load\_command\_A1.m run the 1st, 2nd and 3rd sections
7. Open the PlotPSTHandRaster\_openEphys\_A1.m script in Matlab, Run the 1st section
8. In the 2nd section, modify the following variables:

selectedCodesInd = (1:numel(goodCodes));

selectedCodesIndSpont =[];

1. Run this section and use the created figures to help decide if a cluster is Mua or Noise (in Klusta)
2. In Klusta, go to CorrelogramView, Set window to 1000 and go through the clusters and detect heartbeat noise (6-8 Hz frequency, appears in all channels). Then Set window to 50 and Set bin to 0.5 (ms)
3. Check the displayed refractory period values in the command window in Matlab
4. Go again through all the channels and those with no refractory period mark as Mua
5. Those with a refractory period mark as Good – a good refractory period should have a ratio of <0.125
6. Use Clustering/Recluster or /split to improve the refractory period ratio
7. When done classifying the clusters in Klusta: Clustering/Save; close and clear all in Matlab

Run matlab analysis

1. Run SpikeDataLoading\_openEphys\_A1.m script section by section. Before running the 2nd section, open the variable spikeClusterData.uniqueCodes an type the channel number (from Klusta) in the 2nd column; make sure the channel number in Klusta is correct, sometimes it is displayed wrong
2. Run the other sections till the end of the script
3. In the PlotPSTHandRaster\_openEphys\_A1.m script in Matlab, run the first 2 sections
4. In the 2nd section select the Good/SUA clusters with visual responss and/or optogenetic effect and modify selectedCodesInd and selectedCodesIndSpont (0 for visual-responsive units, 1 for spont.-only units) accordingly and run again the 2nd section
5. Run the next 3 sections
6. Run section 6 (Mua analysis) and select the Mua clusters with good visual response and modify selectedCodesIndMua accordingly and run again the section
7. Run the next 3 sections
8. Run the next section with average of SUA (meanTrace, MeanAllCondTrace.fig) and average of Mua (meanTraceMua, MeanAllCondTraceMua.fig)
9. Open waveformAnalysis\_openEphys\_A1.m and run the first 3 sections