**Guide for spike analysis**

**Data analysis pipeline**

**Copy data**

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

**Preprocess data for Kilosort**

1. In load\_command\_KiloSort\_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 type in the exclude variable the subtrial numbers to be excluded. Run the section
6. In the 5th section choose weather to keep the suggested gain or use a different one (choose between 10, 20, 50 or 100, so that there are not too many data points exceeding 32768) and run the section
7. Run the 6th section
8. Clear variables (clearvars -except experimentName sessionName) and close all figures to empty memory

**Run Kilosort and Phy2 analysis**

1. Type kilosort (after having added its path), fill in the fields
2. Type ks= get(gcf, 'UserData'); ks.ops.fshigh = 300;
3. Press Run.
4. Open the Anaconda Prompt (windows) or Terminal (Linux) in the kilosort analysis folder
5. Type „activate phy2” (Windows) or „source activate phy2“ (Linux)
6. Type „phy template-gui params.py“.
7. Open the SpikeDataLoading\_openEphys\_KiloSort\_A1.m script in Matlab and with the experimentName and sessionName from load\_command\_KiloSort\_A1.m run the 1st, 2nd and 3rd sections
8. Open the PlotPSTHandRaster\_openEphys\_KiloSort\_A1.m script in Matlab, Run the 1st section.
9. In the 2nd section, modify the following variable:

selectedCodesInd = (1:numel(goodCodes));

1. Use the created figures to help decide if a cluster is Mua or Noise
2. In Phy, go to CorrelogramView, Set window to 1000 and go through the clusters and detect heartbeat noise (6-8 Hz frequency, appears in all channels) or other types of noise (optoelectrical artefacts). Then Set window to 50 and Set bin to 0.5 (ms)
3. Go again through all the clusters and those with no refractory period mark as Mua. If necessary, use Clustering/Recluster or /split to get good clusters out of a Mua.
4. Compare the good clusters with each other and decide if they have to be merged or split, also using the traces ploted by Matlab
5. When done classifying the clusters in Phy: File/Save; close and clear all in Matlab

**Run matlab analysis**

1. Run SpikeDataLoading\_openEphys\_KiloSort\_A1.m script section by section. Before running the 2nd section, open the variable spikeClusterData.uniqueCodes and double-check at least for the good clusters the channel number (from Phy) in the 2nd column; make sure the channel number in Phy is correct, sometimes it is displayed wrong
2. Run the other sections till the end of the script
3. In the PlotPSTHandRaster\_openEphys\_KiloSort\_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 section
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 section
8. Run the next section with average of SUA (meanTrace, MeanAllCondTrace.fig) and average of Mua (meanTraceMua, MeanAllCondTraceMua.fig)
9. Open waveformAnalysis\_openEphys\_KiloSort\_A1.m and run the first 3 sections