**Processing headfixed Tytology data**

1. Go through data files and remove any files you don’t want to analyze, including cancelled .dat files. NB: each collected parameter has matching .mat and .dat files – remove both if they exist (cancelled runs have no .mat).
2. Sort all of the data files from one animal so each cell is in an individual folder labeled with the cell ID number and for each file create files necessary for spike sorting in Offline Sorter (OFS) and further matlab analysis.
   1. Move all raw data for single critter into runme folder
   2. Run **OrganiseTytologyDataIntoSubfoldersOfCellNumber.m** . For each cell, this will:
      1. create folders with cell ID # and move files into appropriate folder
      2. convert raw traces to .nex file for use with OFS
      3. create \_cellinfo.mat file for future matlab analysis
   3. Move individual cell folders from runme folder back to appropriate critter folder – final path for each cell should be Data\_Files\Group\Animal\_ID\cell\_ID
      1. (i.e. - Data\_Files\CTR\_Juv\M449\_CTR\_juv\001)
3. Start Plexon Offline Sorter and import .nex file
   1. File – Import – NeuroExplorer file
4. Export to new .plx file – because importing the .nex file only imports a small amount of the trace.
   1. File – Export to New .PLX
5. Sort using Offline Sorter (**see instructions below**).
6. After sorting, export to new .plx again and change file name to “AnimalID\_CellID\_SORTED.plx”
   1. Store in OFS\_Files subfolder
7. Export to .mat file
   1. File – Export Per-Waveform Data
      1. Format = Matlab file
      2. Check Export Unsorted Waveforms
      3. What to export for each waveform – choose only ‘Unit’ and ‘Timestamp’
   2. Hit Export!
      1. Creates AnimalNum\_CellNum.mat file (e.g. F901\_006.mat)
8. Decide whether or not you want to include unsorted spikes when further processing the data.
9. Append spike times from OFS to the .mat files for processing using **AddSpiketimesToMatFiles\_InclUnsort.m**
   1. First, move into runme folder:
      1. data files for that cell
      2. recently created .mat file from OFS
      3. cellinfo.mat file
   2. Run **AddSpiketimesToMatFiles\_InclUnsort.m**
      1. It will ask whether you want to include unsorted spikes or not, and then appends a variable to cellinfo struct (written to cellinfo.mat) indicating whether "unsorted" spikes should be included in downstream analyses (e.g., TytologyPopulationPSTH.m)
   3. This will create a STIMTYPEnew.mat file for each stimulus protocol that was run (‘new’ is shorthand for ‘spikes’ – will be renamed later).

**Matlab processing:**

(Spikes are already appended to a STIMTYPEnew.mat file, and cellinfo.mat knows whether to include unsorted spikes.)

1. Run **movefilesbystimulustype.m**. First need to set the following variables at beginning of script:
   1. Direct startloc to animal folder containing subfolders of different cells, each of which contains many different types of data files (different stimtypes).
   2. Set group with either ‘CTR\_Adult’ or ‘CTR\_Juv’ depending on animal group – stim+destloc and cellinfo\_destloc will use this to set appropriate destination for stim and cell info files
   3. The script will create subfolders in the DestFiles folder for each stimulus type if does not already exist (e.g. DURATION, SIGNALMASK, etc.). It will COPY (not move) two file types into subfolders corresponding to stimulus type (.new.mat and .dat for each set of trials).
   4. This script will also copy \_cellinfo.mat for each cell into the appropriate group depending on animal group (adult vs juvenile). All future analysis scripts will reference this folder to look for cellinfo.mat files
2. Edit **determine\_monotonicity\_updatecellinfo.m** to point to your desired newly-created subfolder (e.g., C:\DestFiles\ABI\, or some other folder where you’ve moved the ABI files you’d like to process).
   1. Sometimes, files are accidentally named FREQ instead of ABI. This script looks at curvesettings.curve.curvetype to determine stimulus type. If there’s a mismatch with the file name, it fixes filenames for the .dat, new.mat, and .mat files.
3. On ABI files (i.e., RLFs), run **determine\_monotonicity\_updatecellinfo.m**. This script:
   1. appends info to curvedata struct for each ABI .mat file (computed values such as meanFR, thrdB, etc.)
   2. outputs summary figure and .txt file for Excel pasting
   3. adds monotonicity information to cellinfo struct (from ABI file curvedata struct)
   4. **JEN, VERIFY THIS IS TRUE:** adds includeUnsort field to cellinfo.mat (user specifies whether to include)
4. (No longer necessary to run **Tytology\_AddToCellinfo.m**)
5. Run **RenameFilesBasedOnParameters.m**, which changes the filenames for GapDetect, SignalMask, and Duration files. (Adds info specific to the parameters, e.g., ToneDur, NoiseDur, FMask, BMask, VarGapDurOnset, etc).
6. Optional but useful: Run **determine\_FSL\_forDuration200ms.m** On duration files for 200ms stimuli, plots distribution of FSL & jitter across all cells (to choose a criteria for well-timed cells). Adds FSL information to cellinfo.mat.
7. Run **PlotStimType.m** on a folder of cells with ONLY a given stimtype (see script for detailed setup instructions):
   1. For each cell and each unit within a cell, creates a page of plots showing
      1. - Rasters of neural responses to each signal (pip duration @ CF)
      2. - PSTHS of those responses
      3. - Mean responses based on relevant measures (FSL, FSL jitter, FR, reliability)
   2. Does t-tests on firing rate and FSL
   3. Saves the figures to activepath/output
   4. Exports these measures to a .txt file in activepath/output (for cutting/pasting into Excel)
   5. Exports the figures to .fig and .pdf files
   6. Creates STIMTYPE\_summary.mat file containing struct out for all cells in folder
8. Maybe just for Duration protocol? Run **PlotTytologyData.m**

For Duration protocols:

1. Maybe just for Duration protocol? Run **PlotTytologyData.m**
2. ResponseDuration\_analysis\_ForStimdur.m
3. post\_respdur\_analysis\_DURATION.m

Other scripts to include above:

**TytologyPopulationPSTH.m**

**Plexon Offline Sorter Spikesorting:**

Required/useful setting-type instructions are RED.

Sequence-based instructions are BLACK.

General navigation instructions are PINK.

1. Open (File-Open) the newly created .plx file.
2. Display trace: In upper right box, double-click on number 1 to select channel (we typically have only one channel, but multielectrode recordings will have more).
3. On bottom left (Timeline box), click Options button and set the following parameters:
   1. Waveform Length = 1311 (32 samples)
   2. Prethreshold Period = 492 (12 samples)
   3. Dead time = 2007 (49 samples)
4. Under Tools – Options, select “Use Only Currently Visible Waveforms”
5. In left control grid, choose clustering type – we’ve been using “K-Means Scan”.
6. *Zoom out along x-axis (buttons for zooming in and out at bottom). Scroll along timeline to see included spikes.*
7. Set threshold that captures spikes but is well above noise floor (in Timeline window).
8. Click Detect (in Timeline window), which detects individual spiketimes based on threshold.
9. Click Find Units (in 2D Cluster window). This sorts waveforms into colored & lettered units.
10. Under Units (middle left), right-click on any noisy units and Delete Selected Unit.
11. Click Control Displays (in Unit window) to only display those traces visible in Timeline below (based on zoom).
12. Choose Center Select (in Timeline window) – now clicking on a point in 2D Cluster window centers that spike in Timeline.
13. *Can click and drag on Waveforms and 2D Cluster windows to see spike shapes.*
14. Scroll, click and zoom around to look at sorted waveforms and decide whether to expand the region in the 2D Cluster window to include more spikes that were defined as Unsorted.
15. To include more spikes, click Add Waveforms (in 2D Cluster window) and draw a circle in that window around the spikes you’ve decided to include.
16. You can combine units (in Unit window) by selecting multiple units, then combining them (right-clicking).
17. Scroll, click and zoom around to decide if you like the sorting results.
18. Remove any obvious movement or stimulus artifacts. How?
19. Try to isolate spike waveforms to one unit, two at most.
20. Note on an Excel spreadsheet:
    1. Threshold (in V)
    2. Whether or not to include unsorted spikes when processing data (you’ll add this information to the cellinfo.mat file using **Tytology\_AddToCellinfo.m**)
       1. If there is clearly a well isolated single unit (as determined by spike waveform) that captures all or most spikes, then do NOT include unsorted
       2. If spikes cannot be reliably attributed to one single unit or separated from each other, include unsorted. ***This assumes that your threshold is set well enough to exclude all noise, so that unsorted waveforms are actually spikes.***