**Data analysis Protocol with Igor Pro**

1. Required installations prior to analysis:
   1. Install Igor pro onto your desktop
   2. Install the “patcher’s power tool” macro to import that data from the .dat file created by patchmaster during recordings.
      1. <http://www3.mpibpc.mpg.de/groups/neher/index.php?page=aboutppt>
   3. Add the following in house written procedure files into the “Igor Procedures” folder of your Igor Pro application folder:
      1. FSCVcal\_Aug19.ipf
      2. FSCVcleaner\_Aug10.ipf
      3. FSCVstim\_Aug19.ipf
   4. Quick note: in house written procedure files written with IGOR pro v6.37, therefore any other IGOR pro version used may require code adjustments
2. Analyze Calibration data
   1. Open the data browser (Data > Data Browser) and ensure the red arrow in the white folder box is on the root folder (fig1a)
   2. Go to the FSCV tab and select “New Calibration Folder” (fig1b) to create a new Calibration folder in your root folder
   3. Transfer the red arrow to the calibration folder in the data browser (fig1a) by using you mouse and then open the calibration with a double click
   4. Then load the current and voltage pulses by using the patcher’s power tool macro. Go to the PPT tab and click “Load PULSE/PM file”
   5. A macro will appear (fig1c), where the analyzer must select desired file, group and series. DO NOT SELECT overlay, this can cause the program to freeze due to the heavy file. Base name isn’t that important for the code, but the analyzer can change the name as desired. Then click “Do it” to start the import process
   6. Next the data must be cleaned using the “Data Cleaner” window. Bring up the window by selecting FSCV> Cleanup data
   7. A window will appear called “Data Cleaner” (fig1d, #1). Select “Organize V-Waves” and the program will delete all voltage waves except for 1 and rename it “CommVolt”. This should be apparent in the data browser (fig1a).
   8. Once that is done, the analyzer can close the “Data Cleaner” window by clicking “Goodbye!” (#3)
   9. Next is the actual electrode calibration. Click on FSCV>Electrode Calibration.
   10. A window named “Calibrator” should appear (fig2)
   11. The current waves must then be redimensioned by clicking on the “Redimension Waves” button (#1) on the top left hand corner of the window. Depending on the amount of waves in the folder will determine how fast or slow this process is
   12. All the current waves should be re-dimensioned and renamed in the data folder (fig1a)
   13. Next the current time course must be visualized for specific wave selection for consecutive steps. To do this, click on “Get Timecourse” button (#2). This can be slightly delayed by the amount of waves present, but eventually the timecourse wave (average current at oxidation vs sweep count) should appear (fig2)
   14. Next, the specific waves for averaging and voltammogram creation must be selected. This can be done by first going to Panel>show info. A thin rectangular panel will appear at the bottom of the “Calibrator” window (fig2, #3a).
   15. Place the corresponding cursors in the desired sections of the timecourse graph as follows (#3): (make sure the amount of current voltage pulses selected for each cursor pair is identical)
       1. A/B=baseline
       2. C/D=1st DA concentration
       3. E/F=2nd DA concentration
       4. G/H=3rd DA concentration
       5. I/J=4th DA concentration
   16. Once the cursors are placed in the desired sections of the timecourse graph, click on the “Calc Voltammograms” button (#4). This will calculate the average current pulse for each concentration, create background subtracted pulses and graph each one against the command voltage (fig2)
   17. The maximum current produced at oxidation (~600mV) must next be calculated. Click on the left bottom panel in the “Calibrator” window so the info panel applies to it and place the A cursor to the left of the peak and the B cursor to the right of the peak (#5).
   18. In the black outlined box in the bottom right portion of the “Calibrator Window” enter the numerical DA concentration (in µM) in the boxes right under “Known concentration (uM):” text (#6). Click on the “Calc Calibration Curve” button right (#7) above the black outlined box and the calibration curve should appear in the panel right to the left of it. The sensitivity and the calculated error should also appear within the black outlined box
   19. Outside the “Calibrator” window, duplicate voltammogram and calibration curve graphs should appear along with a table of DA concentration vs current amount. These graphs and table can be manipulated as desired for exportation.
3. Analyze slice stimulation data
   1. Follow steps 2a-g except for the stimulation folder with the respective loaded data
   2. Since an unnecessary amount of current pulses must be recorded during slice stimulation for signal stabilization, a large amount of current pulses must be deleted to make data handing easier. There are 2 input boxes labeled “first pulse” and “last pulse”, type in the pulse number range that should be deleted (fist and last pulse) and click on “delete current pulses” (fig1d, #2).
   3. Once the undesired current pulses are deleted, close out the “Data Cleaner” window by clicking on “Goodbye!” (#3)
   4. Next bring up the “Stim Analysis” window by going to FSCV>Stimulation Analysis.
   5. The current waves must then be redimensioned by clicking on the “Redimension Waves” button (#1) on the top left hand corner of the window.
   6. All the current waves should be re-dimensioned and renamed in the data folder (fig1a)
   7. Next the current time course must be visualized for specific wave selection for consecutive steps. To do this, click on “Get Timecourse” button (#2). The timecourse wave (average current at oxidation vs sweep count) should appear (fig3)
   8. If the analyzer desires to calculate background subtracted current waves in bulk, they may do so by first using the cursers (Panel>show info) to identify baseline and the region of interest. Place cursers A and B at the end and beginning of the baseline (prior to stimulation on timecourse graph). Then place cursers C and D at the beginning and end of region of interest after stimulation (#3). Once the cursers are set, click on “Subtract Background Current”(#4). There will be some delay due to heavy processing but the subtracted waves should appear in the data in the data browser (fig1a)
   9. Next the DA peaks must be normalized to baseline. In the top graph timecourse panel, verify that cursors A and B are at the beginning and end of the baseline, respectively. And place curser E at the beginning of baseline and cursor F at the end of the region of interest (#3). Click on “Graph Normalized data” in the middle of the “Stim Analysis” window and a normalized timecourse should appear in the bottom panel (#6).
   10. A duplicate normalized timecourse graph is created outside the “Stim Analysis” window for export purposes along with a table with the normalized DA peak value for the baseline along with the region of interest.