Advanced Electrophysiology 12 March 2025 - 2 April 2025

1) **Download Clampfit** 11.4.1 (or previous version if you do not have Windows 10 or 11) at:

https://support.moleculardevices.com/s/article/Axon-pCLAMP-11-Electrophysiology-Data-Acquisition-Analysis-Software-Download-Page

google: clampfit download

(Windows only; for mac users you need parallels, available at

https://www.parallels.com/

14-day free trial available

2) **Transfer of set of data for analysis**

In the Electrophysiological data folder, you will find 10 folders named KO Cell1-5 and WT Cell1-5. They contain electrophysiological recordings from 5 WT and 5 I*tgb3* KO layer V pyramidal neurons from medial prefrontal cortex acute slice of mice. *Itgb3* is an ASD (autism spectrum disorder) gene expressing the cell-adhesion molecule β3 integrin localized specifically in layer V pyramidal neurons. Each folder contains 3 files that you can open with Clampfit. They are sequential recordings of excitatory synaptic currents from the same layer V pyramidal neuron:

1) Baseline: Baseline recordings (12 traces recorded every 20 s)
2) Drug: 36 traces (every 20 s) during application of MPEP (20 µM; 2-Methyl-6-(phenylethynyl)pyridine hydrochloride), a non-competitive antagonist of the metabotropic glutamate receptor mGluR5
3) NBQX: 20 traces (every 20 s) during application of NBQX (10 µM; 2,3-Dioxo-6-nitro-1,2,3,4-tetrahydrobenzo[*f*]quinoxaline-7-sulfonamide) a competitive AMPA and kainate receptor antagonist.

3) Opening and visualization of the traces with Clampfit

a. Select sweeps
b. Adjustment visualization window
c. Brief overview of the analysis possibilities
d. Sampling interval (20 µs; 50 kHz)

4) What do the traces show:

a. Voltage command: holding potential: -70 mV, passive properties, voltage-gated currents, mEPSCs
b. Stimulus artefact, AMPAR synaptic currents (recordings done in the presence of Bicuculline (10 μM) a competitive $GABA_A$ antagonist and D-APV (50 μM; D-2-amino-5-phosphonovalerate), a competitive NMDA receptor antagonist.
c. How to isolate currents in native systems (voltage command, pharmacology, KO) as opposed to heterologous expression systems.
d. 50 Hz noise

5) How and why the experiment was done

a. AMPAR excitatory synaptic currents (amplitude, rise time, decay time)
    i. Does ablation of β3 integrin affects AMPAR excitatory synaptic currents under basal conditions
    ii. Does ablation of β3 integrin affects the mGluR5-mediated regulation of AMPAR synaptic currents (rational: mGluR5-mediated LTD; mGluR5 theory of fragile X-syndrome)

b. Paired pulse ratio as a proxy of synaptic vesicle release probability (P_r)
    i. Does ablation of β3 integrin affects P_r under basal conditions
    ii. Does ablation of β3 integrin affects P_r upon blockade of mGluR5

c. Even traces: holding potential: +40 mV
    i. Does ablation of β3 integrin affects the AMPAR rectification index under basal conditions
    ii. Does ablation of β3 integrin affects the AMPAR rectification index upon blockade of mGluR5

6) How to analyze the data

a. How to average traces
b. How to offset traces
c. How to calculate curent amplitude
d. How to calculate current rise time
e. How to calculate current decay time constant: the weighted decay time constant
f. How to calculate AMPAR PPR
g. How to calculate AMPAR rectification index
h. How to calculate the effect of MPEP
i. NBQX: how and why to digitally subtract traces

7) **Install Igor Pro and GraphPad Prism 5 on your system**

8) **Using a computer routine to automate data analysis**
   - Passive properties: R_s R_{in} and C_m

9) **Using Two-way ANOVA – Bonferroni post-test**

	WT	KO
Baseline		
MPEP		