MODULE 1 assignments

In vitro patch-clamp recordings and data analysis

(Vanessa Stempel, Chryssanthi Tsitoura, Oriol Pavon)

The aim of these sessions is to obtain whole-cell patch-clamp recordings from neurons in acute brain slices, and to use this technique to measure some biophysical properties and synaptic integration. You will also be provided with some data for analysis, in the unlikely case that you can't obtain data by yourselves. Analysis will be done in a Python environment, or in your favourite alternative if you have one.

1 - **Basic biophysics**

a) obtain a cell-attached recording in VClamp, and measure the seal resistance (tip: use the command potential and the holding current)
b) go into whole-cell configuration in VClamp, and measure access resistance and whole-cell capacitance by compensating the capacitive transient
c) go into IClamp, and measure the resting membrane potential
d) in IClamp, use current steps to make the neuron fire and plot a FI curve. Using the same data, estimate mTau. What is the most appropriate current step size to do this?
e) in VClamp give voltage steps and plot IV curves for different parts of the step. What currents can you identify?
f) in VClamp, give a large voltage step preceded by a step of increasing magnitude. What happens to the currents? Plot the peak current as a function of the pre-step voltage.
g) for extra points, isolate some of the currents pharmacologically. What blockers should you use?

2 - **Synapses**

a) in VClamp record spontaneous activity at different holding potentials. Analyse the data by first extracting synaptic events for each holding potential and making an average waveform. What do you observe? Can you detect any inhibition? For further analysis on the average waveforms, measure the rise and decay time. Try also to measure the peak and rise time of each individual event and plot histograms of these data. Plot the peak against the rise time - is there any correlation?
b) do the same experiment in IClamp and extract average waveforms. Compare the rise time and the decay of the synaptic events recorded in IClamp and VClamp. Are they different?
c) try to test for the presence of NMDA receptors without using pharmacology

3 - **Optogenetics**

a) record from a cell that expresses ChR2, and in VClamp deliver light pulses of different intensity to obtain and current-light intensity relationship. Switch to IClamp and do the same thing to obtain and AP firing-light intensity relationship
b) in VClamp, deliver two light pulses and gradually increase the time interval between them. What can you observe? How many current components can you see in a single light pulse?
c) Try to determine the fastest frequency at which you can reliably use ChR2 to elicit currents and action potentials
d) record from a cell that does not express ChR2, and deliver a train of light pulses at a frequency informed by the previous experiment. Can you see any light-evoked postsynaptic responses? If so, vary the stimulation frequency in VClamp to measure the short-term plasticity properties of the connection. Do the same in IClamp - does the cell fire? How does it depend on the stimulation frequency? Can you tell if it is a monosynaptic connection? Is it excitatory, inhibitory, or both?

In vivo patch-clamp recordings and data analysis

(Mateo Velez-Fort and Christopher Puhl)

The practical will demonstrate the utility and the application of *in vivo* whole-cell recordings in anaesthetised mice. Students will obtain data recorded from the primary visual cortex while rotating a vertical bar around the animal's monocular and binocular visual fields.

A brief introductory tutorial will cover the technical aspects and its application to *in vivo* experiments. Students will observe animal surgery and preparation for electrophysiological recording (Tuesday afternoon and Wednesday morning) and develop an intuition for experimental design and key aspects for successful setup and recording. Finally, this practical will involve analysing stimulus-evoked responses. An introduction to Python and the challenges related to analysis language and data types will provide guidance of how the data will be analysed. Students will then analyse the electrophysiological data using Python scripts to provide a quantitative description of the physiological response properties of recorded neurons.

NEURON tutorial

(Lee Cossell)

The goal of this assignment is to use the NEURON simulation environment to explore the biophysical properties of neurons. We will run simulations to investigate the parameters that determine passive properties, the basic dynamics of HH-type channels and action potentials, and the main determinants of synaptic integration efficacy.

1 - **Passive properties**

a) Make a ball model and measure Ri, mTau with current injection
b) Change diameter, Rm, Ra, Cm and see how it affects Ri and mTau
c) Add dendrites and axons of increasing total areas. What happens to Ri and mTau?
(pay special attention to whether mTau is a single exponential)

2 - **Active properties**

a) Add HH Na and K voltage-gate channel to the ball model and inject current steps
(add one at the time to see the effect of each one, and then combine)
b) Measure gNa and gKv as a function of current injection at the soma, and plot activation curves (from resting Vm)
c) Measure gNa and gKv to a large current step from increasing resting Vms, and plot inactivation curves.
d) Give two large current steps separated by an increasing time interval, and plot gNa. What happens?
e) Play with gNa and gKv density to make an AP
f) Add an axon and try to make the AP start in the axon
g) Add a dendrite and measure the AP signal in dendrite
h) Add voltage-gate Na channels to the dendrite and see how if affects the AP signal (use different total dendritic areas and branch lengths)

3 - **Synaptic integration**

a) Add a single alpha excitatory synapse in the soma and then in the dendrite, progressively far away, and record at the soma. What changes?
b) Do the same but record at the dendrite where the synapse is. How does it compare to the soma? What changes with distance?
c) Change Rm, Cm and Ra - how does it affect the EPSP locally and at the soma?
d) Add 10 excitatory synapses and activate them with increasing temporal intervals in a soma only model (do this for passive and active models, plotting EPSP peak and AP number as a function of inter-synaptic interval). How does the temporal integration window depends on mTau?

e) In a model with dendrites, compare the somatic EPSPs generated by 10 synapses in the same dendritic location versus 10 synapses in different dendritic branches.
f) Repeat e) in the presence of NMDA receptors. What changes?
g) Can you get a sodium spike in the dendrites? And an NMDA spike? Where is it easier to get one?
h) In a passive ball model, add one inhibitory and one excitatory synapse, and activate both at different time intervals. When is inhibition more effective? How does this depend in the reversal potential of the inhibitory synapse?
i) In a passive model with dendrites, place the excitatory synapse in the dendrite, and vary the location of the inhibitory synapse (including placing it distal or proximal to the excitatory synapse, and in a different dendritic branch). What configuration is more effective for inhibiting excitation?