BBB492 Experimental Methods in Synaptic Physiology

Instructor: Mike Kaplan

Wednesday, 2-5 pm LEIDY12 (Neurolab)

This course an introduction to electrophysiology using three invertebrate preps: the neuromuscular junction the tail of the crayfish *(Procambrus clarkii)*, buccal ganglion in the freshwater snail *(Helisoma trivolvis)* and the abdominal ganglion of the marine gastropod, *Aplysia californica.*

After a week devoted to getting to know the equipment, making sure everything is running smoothly, and learning troubleshooting and maintenance to keep it that we will spend two weeks on each of the three preps. As we finish with each prep, you will collect whatever data you recorded and send me a brief summary showing samples of your recordings and analysis when appropriate. We will also try to start every other class with a “data roundup”, where we will briefly show and discuss our best data from the previous two weeks. You should also maintain a lab notebook with all your data for the semester- we will go over how to do this and just what information needs to be included for an experiment to be subject to meaningful analysis. At the end of this six-week trial period (Week 8), we will present highlights of our data to the class – nothing too elaborate, just some representative waveforms and the relevant analysis.

In Week 8 we will also pick the preps and the particulars of our final projects. You first need to decide which prep you want to work on (usually determined by the prep where you had the most success generating data). Some people turn out to be very good on the crayfish prep, others prefer the *Aplysia* or snail preps. Attached is a list of possible projects for each of the three preps- you are of course entirely free to think up your own projects that go beyond this brief list. The last six weeks of the course are devoted entirely to working on the final project, which will be described in a short paper to be turned in on the last day of finals week (May 8).

*Date Lab*

Jan 9 Check all components and tools, and test the rig using model cells. Learn to fill electrodes and check electrode resistance. Learn to solder, and other troubleshooting issues. Learn to use Chart software to view and record, export data and keep an ongoing notebook of collected data. *Reading for next time:* *Crawdad manual, labs 2 & 4 and Appendix A (available on Blackboard: Documents/MANUALS and GUIDES).*

Jan 16 CRAYFISH 1: Intracellular recording from tail muscle in *Procambrus clarkii* using sharp electrodes. Learn to prepare crayfish tail NMJ prep. *Reading for next time:* *Crawdad manual, chapters 8 and 9.*

Jan 23 CRAYFISH 2: Extracellular recording from the NMJ using suction electrodes. Learn to construct and use suction electrodes to record from or stimulate a nerve, using same the same tail prep from the previous week. Try to get a simulataneous recording from nerve and muscle. *Reading for next time:* Murphy 2001.

*We may stick with crayfish for one additional week, depending on how we do as a class- if so, all dates below will be moved back one week*

Jan 30 SNAILS 1: Intracellular recording from buccal ganglia in *Helisoma trivolvis.* Use intracellular recording technique from weeks 2 and 3 on identified neurons in the rhythmic neural network. *Reading for next time:* *continue reading Murphy review.*

Feb 6 SNAILS 2: use pharmacological agents to help identify neurons. Work on the perfusion system to get a good flow through the chamber without running dry. *Reading for next time: Aplysia lab manual.*

Feb 13 APLYSIA 1: the abdominal ganglion of *Aplysia californica* contains the circuitry responsible for the gill withdrawal reflex, the subject of extensive studies relating synaptic plasticity to learning. Using the sharp and intracellular electrode techniques from the previous weeks, find the synapse from the gill and siphon sensory neurons to the motor neurons controlling the withdrawal response- the synapse that changes when the withdrawal response is subject to conditioning.

Feb 20 APLYSIA 2: Now, do it again.

Feb 27 Data roundup, rig maintenance, discussion of final projects (see list on next page).

SPRING BREAK

March 13 Final projects

March 20 Final projects

March 27 Final projects

April 3 Final projects

April 10 Final projects

April 17 Final projects

1.  *Aplysia* projects

a. SN:MN synapse of the siphon withdrawal reflex: this is the synapse that is modified in sensitization or classical conditioning of the reflex, the very synapse that put Eric Kandel on the map. It is relatively easy to access: the axons of the unipolar sensory neurons (the LE sensory neurons) run through the siphon nerve (the “extra” or 5th nerve coming out of the abdominal ganglion, the one that doesn’t seem to come out of one of the four corners.) A suction electrode on the siphon nerve allows you to initiate action potentials in these cells. They make synapses on the LFS motor neurons that innervate the muscles of the siphon. You can hit these cells with a sharp electrode- and the way to know that you have the right cells is that you can observe the EPSP through your sharp electrode each time you initiate an action potential with the suction electrode. Being able to find this synapse is the lynchpin of many possible experiments- for example, the effect of serotonin on this synapse. The LFS cells are fairly small but the criteria described above for identifying them correctly are particularly clear-cut.

b. You should also be able to find the cell bodies of the LE sensory neurons while initiating action potentials in their axons using the same suction electrode on the siphon nerve. They look like a little bunch of grapes, and siphon nerve axons lead right to them. This means you could study spike propagation at high frequencies (ii.e., is there a frequency above which the action potentials initiated in the siphon nerve do not propagate safely back to the cell body? Does serotonin (which blocks several different K channels) relieve spike propagation failures?

c. If you want to study synapses but don’t want to deal with the suction electrode, put an electrode in one of the LE sensory neurons (the aforementioned bunch of grapes), and then put a second electrode in one of the LFS motor neurons. The LE neurons should be easily identified, and a monosynaptic EPSP in the second neuron confirms that it’s one of the motor neurons.

d. R15 is another interesting cell that has been the subject of many publications and studies on the mechanism of its bursting waveform. More generally, almost any identified neuron in *Aplysia* can easily serve as the subject of a final project, provided the criterion for identifying the neuron are clear enough

e. *Aplysia* buccal ganglia: this is largely unexplored territory in the Neurolab. It would be very, very interesting to record some rhythms from the *Aplysia* buccal ganglia to compare to their counterparts in *Helisoma* (snails).

Crawdad projects:

a. Best project with this prep is to get some simultaneous nerve and muscle recordings using suction and sharp electrodes. Sort the spikes by size, and see which class of spikes has a constant latency to the EPSPs in the muscle.

b. Once you have the dual recordings, you can cut the nerve near the notochord, and use the suction electrode to stimulate the siphon nerve and fire the synapse at particular patterns. Could thus explore synaptic properties like paired pulse depression or facilitation (PPD or PPF), post-tetanic potentiation (PTP), or even LTP.

*Helisoma* projects

a. Application of the suction electrode to some of the nerves coming out of the buccal ganglia presents several different possibilities for a project. Simply obtaining a number of these recordings, from several different nerves, would be a good and useful project.

b. Since a suction recording electrode is also a stimulating electrode, we can look at the effect of stimulating some of these nerves coming out of the ganglia on the rhythm of the ganglia, while recording from intra or extracellular electrodes (you can stimulate with one suction electrode while recording from up to two additional ones.) Some of the incoming nerves contain axons of projection neurons that release neuromodulators like dopamine and serotonin as well as peptide modulators, which enable the core rhythm of the buccal ganglia to be modified. Some of these effects should be mimicked by stimulating the right nerve trunk.

c. *Helisoma* also displays some interesting pharmacology, but our ability to explore it has been limited by the lack of suction for perfusion. There are various ways to address this issue. Getting some kind of perfusion system running and characterizing the effects of one or two drugs like dopamine, serotonin and their agonists/antagonists would be a fine project (especially for tinkerers).