

BIOS 442 ASSIGNMENT TO BE DONE IN DISCUSSION/LAB SECTION

Launch the NMJ v2.0 application from the desktop

1)

Click on help and read through all four contents sections.

Return to the main screen

We will investigate the effects of altering presynaptic Ca^{2+} entry to the neuromuscular junction and then postsynaptic processes.

2)

Under STIMULUS click on the button labeled STIMULATE NERVE, then hit DO SWEEPS.

This represents the normal electrical response recorded in a muscle cell to the stimulation of an action potential in the prejunctional compartment of the neuromuscular junction.

What electrical components does the trace represent?

3)

Click on IONS.

Change Mg to 8mM Click OK. What will the effect of this change be on Ca^{2+} entry to the prejunctional Ca^{2+} entry and why?

Click DO SWEEP.- Repeat this a number of times and observe the response. Does the stimulus always evoke a response?

Position the vertical cursor (gray line that passes from top to bottom of the view area) before the evoked response (at $T=0.1$ to about 0.3ms). Stimulate with DO SWEEP once or twice to ensure that there is no spontaneous response at the point measured. Record (Write down) V_m .

Reposition the vertical cursor at the peak of the response (about 0.75ms). Click DO SWEEP about 200 times and record V_m at the peak of the evoked response each time. Note that sometimes no response will occur. Still record the amplitude which should be more or less identical to the V_m recorded prior to the stimulus.

Homework assignment

Calculate the difference between each of these 200 results and the V_m recorded prior to the stimulus. Bin these responses and construct a histogram of amplitude vs number of events.

(Tips – You are looking for a result similar to that seen in Kandel Schwartz and Jessel fig 14.6.

The look of the end result is very sensitive to the size of the amplitude bin.

This data can be binned very quickly using the data analysis pack in microsoft Excel.)

Do these responses vary smoothly or in distinct jumps? Why?

4)

Click on IONS Click RESET to NORMAL. Click OK

Under STIMULUS click NO STIMULUS then DO SWEEP.

Do you see any events? Of what amplitude? How does this relate to the amplitudes of the stimulated events that you saw in section (3) above?

5)

Click DRUGS Click TUBOCURARINE. For the concentration add $5 \mu\text{M}$ (ie $5\text{E-}6\text{M}$) or $5 \times 10^{-6}\text{M}$. Click OK and then DO SWEEP. If you repeat this over and over what happens to the response amplitude? Do you ever see no response? How does this compare to when you raised the Mg^{2+} concentration previously?

What do you think TUBOCURARINE does (where is it acting) and why do you think this?