

NEUROBIOLOGY LAB Fall 2009

EXPERIMENT #3: The neuromuscular endplate potential (EPP).

In this experiment, you will use the same frog sciatic neuromuscular preparation that was used for Exp #2. Refer to the description of that experiment for details related to the anesthesia, dissection and electrophysiological configuration. Much of the METHODS section of your report for Exp #2 will also apply for this experiment. The main difference will be your procedure which is now designed to study the synaptic endplate potential.

Stimulation of the hundreds of nerve fibers in the sciatic nerve evokes action potentials which generate the compound action potential (cAP). These action potentials propagate to the neuromuscular junctions which are synapses that release the neurotransmitter, acetylcholine, and open excitatory channels on the endplate of the muscle fibers. This generates a postsynaptic potential on the muscle called the endplate potential (EPP) which is similar to the excitatory postsynaptic potentials (EPSPs) generated by neurons.

The specific objectives of this lab will be:

1. Plot the input/output response of the endplate potential (EPP) as a function of the compound action potential (cAP).
2. Examine a short-term form of synaptic plasticity called paired-pulse facilitation.
3. Determine the upper following frequencies for both the cAP and the EPP.

Adjust the stimulator for a minimum pulse duration (0.02ms) and a voltage that gives a near maximal cAP amplitude recorded at the site along the nerve that is nearest to the muscle. To record the EPP, you may need to move the recording clip to the site near the middle of the muscle. You will need to increase the horizontal axis of your computer graph to see the EPP. Notice the significant delay between the cAP and the EPP and the much longer duration of the EPP. The response should look something like Figure 1.

FIGURE 1. The Neuromuscular Endplate Potential (EPP).



PART I: Plot the input/output response of the neuromuscular junction.

Across a range of stimulus intensities from subthreshold to supramaximal, measure the amplitude of the cAP and the EPP. The peak amplitude of the EPP will be measured the same way you measured the peak amplitude of the cAP. It may be necessary to move the recording clip back and forth between the nerve (to record the cAP) and the muscle (to record the EPP).

Using the minimal pulse duration (0.02ms), gradually increase the voltage to the threshold for the cAP. Is there an EPP at the cAP threshold? Continue to increase the voltage beyond the cAP threshold until you record a just-measurable EPP. This voltage is the EPP threshold and it may be slightly greater than the cAP threshold. NOTE you may specify the EPP threshold either as the stimulus intensity OR as the minimum cAP that evokes an EPP. (Why is this cAP measure of the EPP threshold better?) Continue increasing the voltage gradually, and make a table of the peak amplitudes of the cAPs and EPPs associated with each stimulus voltage.

FOR YOUR LAB REPORT: Include a **plot of five superimposed graph traces** demonstrating the range of the response from EPP threshold to EPP maximal response.

FOR YOUR LAB REPORT: Include **two graphs plotting the input/output response**. The first should plot the cAPs and EPPs as a function of stimulus voltage. This will look like Fig. 3 of Exp #2 with two curves rather than one. Remember to indicate the pulse duration on your graph. The second graph should plot the amplitude of the EPP as a function of the cAP amplitude. This second graph shows the input/output response of the neuromuscular junction. What does this second graph add that is not obvious in the first?

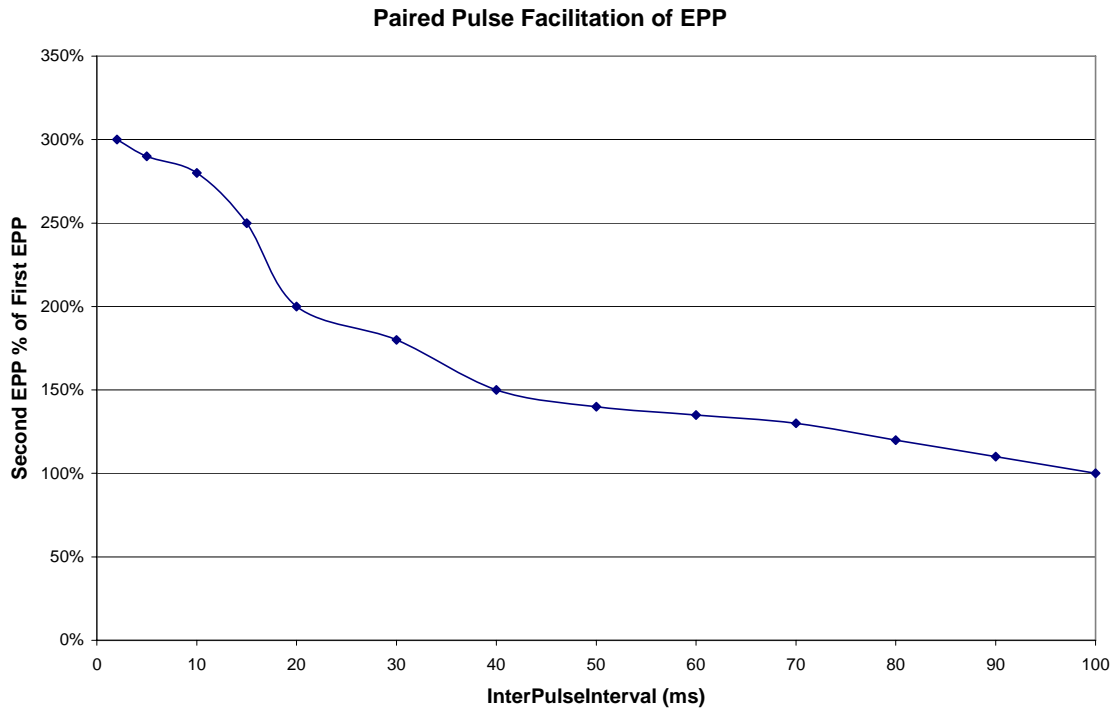
PART II: Examine Paired-Pulse Facilitation.

Set the switch on your stimulator to deliver TWIN PULSES and increase the DELAY to 10 ms. Now when a stimulus is triggered, the stimulus artifact occurs at the very beginning of the graph trace and after the time set by DELAY, a second stimulus artifact appears. You may need to further increase the horizontal axis length to get both EPPs evoked by the first and second stimuli to appear on your graph. Notice that the second EPP is not the same amplitude as the first. This difference between the first and second EPPs is due to **paired-pulse facilitation** which is a simple form of short-term synaptic plasticity.

To examine this phenomenon, set the stimulus pulse duration to the minimum (0.02 ms) and the voltage to evoke a **just** maximal EPP (not too strong). **DO NOT CHANGE THE STIMULUS THROUGHOUT THIS PART OF THE EXPERIMENT.** Trigger a stimulus and measure the amplitude of the first and second EPPs. Then increase the stimulator DELAY setting and trigger another stimulus. For this experiment you will continue to increase the delay and measure the amplitude of the second EPP. **ON EACH RESPONSE, THE AMPLITUDE OF THE FIRST EPP SHOULD REMAIN CONSTANT (why?).**

FOR YOUR LAB REPORT: Include **one plot of superimposed traces** for each delay increment of 10 ms.

FOR YOUR LAB REPORT: Include a **table of second EPP amplitudes** and a **graph** of the second EPP/ first EPP ratio as a function of interpulse delay. This graph should look something like Figure 2:



PART III: Find the maximum following frequency for the cAP and EPP.

Turn the stimulus frequency down to one pulse per second and switch to REPEAT. Both the cAP and the EPP should respond to each stimulus. Now turn off the stimulus and increase the frequency to 5 pps. Briefly switch to REPEAT while watching the response on the graph. Notice that the EPP grows slightly and then reaches a steady response. Repeat this procedure with greater and greater frequencies until the EPP can no longer keep up with the stimulator. This stimulus frequency is called the maximum **following frequency** for the EPP. Now repeat the procedure while recording the cAP and find the maximum following frequency for the cAP. Note that the maximum following frequency for the cAP is much greater than that for the EPP.

FOR YOUR LAB REPORT: Include **one plot of the response** that demonstrates an intermediate frequency that is below the following limit for the cAP but above the following limit for the EPP.

FOR YOUR LAB REPORT: **State the maximum following frequencies** for the EPP and the cAP in your RESULTS section and comment on the meaning of this finding in your DISCUSSION. Note whether the EPP recovered to its original amplitude after the high frequencies and discuss why.

REMEMBER FOR YOUR LAB REPORT:

- Describe the experiment in the **past tense**.
- Start with a good TITLE and put your name on the paper.
- The ABSTRACT should summarize **why** we did the experiment, **how** we did it, **what** we found, and the conclusion. This short section is worth as much as the others.
- In the INTRODUCTION section, state the purpose and aims of the experiment. This section should include enough background only to explain what problem the experiment addressed or why the specific technique was used.
- In the METHODS section, do not include minor details such as the stimulator settings or the color of the connection wires. Also, do not refer to data figures in the METHODS.
- In the RESULTS section, refer to each data figure or graph figure and describe in words what they show. Remember to specify the actual values of results such as conduction velocity or following frequency **in words**.
- In the DISCUSSION section, start with a summary of your findings and state the meaning of these findings as your conclusion. Also, identify possible sources of error in your data collection or measurements and suggest ways to improve. Finally, suggest future experiments that may add to your knowledge.
- The REFERENCE section should only include items that were specifically cited in your paper. In the paper, the citations should look like: "Cauller and Kulics, 1991" or "Kandel *et al.*, 1991" if there are more than two authors. The complete reference is then listed in the REFERENCE section in alphabetical order.