

Killing George: Refractory Effects at Low Stimulation Amplitudes

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Abstract

We investigated the refractory effects due to low pulses of electric current on the sciatic nerve. Based on previous studies, we expected that a pair of identical stimulus pulses separated by a delay less than the refractory period would produce two CAPs where the second CAP would be attenuated. We hypothesized that as the magnitude of the stimulus pulses decreased, the magnitude of the second CAP would increase until it equaled that of the first CAP. In the lab, we measured and recorded the CAPs that resulted from pairs of stimulus pulses separated by less than the relative refractory period for several different stimulus amplitudes. Our results suggest that as stimulus amplitudes decrease, refractory effects become negligible, but more research is needed to verify these results.

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1 Introduction

The sciatic nerve is a sensory and motor nerve originating in the spinal cord and running through the pelvis and leg. The cells in the nerve are electrically excitable, so when an electric current above the cell's threshold level is passed through the cellular membrane, an action potential (AP) is generated. The superposition of the action potentials produced by the individual nerve fibers in the same nerve is called the compound action potential (CAP).

Nerve cells are refractory, meaning that immediately after one AP there is a delay before a second AP can be generated. If the interval between pulses is less than the absolute refractory period, it is impossible to generate a second AP at all, regardless of how high the intensity of the stimulus is for the second pulse. For intervals slightly longer than the absolute refractory period, there is a relative refractory period. During that time, a second AP can be generated, but its threshold is elevated. The threshold for eliciting the second AP during the relative refractory period depends on both the history of previous stimulation and characteristics of the responses of the particular axon [1].

Each nerve cell has a different threshold value that is described by a probability distribution [2], so a stimulus pulse of a very low intensity triggers a small fraction of nerve fibers. When using two identical electric pulses of a low magnitude separated by a time interval within the relative refractory period, we expected that the second pulse would not elicit any response from the small number of fibers that triggered the response for the first pulse. Their threshold levels would be higher due to the effects of the refractory period. However, the vast majority of the nerve cells would not be refractory for the second pulse, and this second pulse would trigger some of those cells. As a result, we hypothesized that the first CAP response would look nearly identical to the second CAP response for low stimulation amplitudes.

2 Methods

The sciatic nerve was removed from a bullfrog following the procedure described in the lab manual [2]. We completed the basic observations outlined in the manual, including measuring the threshold voltage and identifying response components.

2.1 Finding the Refractory Period

We first measured the refractory period of the nerve. We used two identical electrical pulses, each of 100 microsecond duration repeated at a rate of 10 per second with an amplitude of slightly more than the measured threshold voltage. The time interval between the two pulses was increased until there were indications of a second CAP response. The absolute refractory period was the time period from 0.0 ms until when the second CAP response became visible. To find the end of the relative refractory period, the time interval was further increased until the second response was identical to the first response in shape, amplitude, and width.

2.2 Finding the Minimum Intensity Threshold

After we had determined the length refractory period, the sciatic nerve was stimulated with repeated pairs of pulses. The two pulses in each pair were identical in magnitude and duration. The duration remained constant at 100 ms. The pulses were separated by a time interval less than the relative refractory period measured above. In each of these measurements, the stimulus pulses were separated with a delay of 7.4 ms, which was as close to the refractory period as we were able to get before the pulse delay became so small that the second stimulus interfered with the first response.

Measurements were taken with stimulus amplitudes ranging from 0.75 mV to 1.2 mV at 0.05 mV intervals. The minimum intensity threshold level was the magnitude of the stimulation below which there was no visible second CAP response. After taking those measurements, we measured the absolute and relative refractory periods for a second time, following the same procedure described in 2.1, as a control.

2.3 Analyzing the data with Matlab

Finally, we analyzed the data collected in the lab using Matlab. To expedite this analysis, we used a modified version of the Matlab software packages provided for the course. For each response measurement, we measured the amplitude of the response to both pulses, as well as the times at which those responses occurred. Our amplitude measurements included the height of both phases of the CAP. For each CAP in each measurement, we calculated the response height by taking the difference of the minimum and maximum voltages measured during the time period corresponding to that CAP. We then calculated the ratio of heights of the two responses observed.

We also devised a technique to compare measurements of nerve responses to pulses of different amplitudes. This involved normalizing the second measurement so that the heights and offset of the first peak in both measurements were aligned in both voltage and time.

3 Results

After measuring the refractory period, we captured graphs of how the sciatic nerve responded to pairs of identical stimulus pulses. We conducted 11 measurements, each time varying the pulse amplitude. Due to experimental errors, we had to discard the last four measurements.

The time interval used in each of these measurements was only slightly below the end of the relative refractory period because we ran into a problem: For very short time intervals, the generation of the second pulse interfered with the results of the first pulse. The maximum height of the first impulse could not be recorded due to over-lapping with the second pulse's artifact response. At the time interval we chose, there was no significant interference between the response due to the first pulse and the generation of the second pulse.

Figure 1 shows our measurements with stimulus amplitudes of 0.9 mV (solid line) and 1.2 mV (dashed line). In this plot, the 1.2 mV measurement has been scaled and shifted so that it matches the 1.2 mV measurement. Figure 1 clearly shows that the normalized

Stimulus Voltage (mV)	First Peak Height (mV)	Second Peak Height (mV)	Height Ratio
0.9	0.147	0.141	0.959
0.95	0.302	0.244	0.808
1.0	0.431	0.369	0.856
1.05	0.54	0.437	0.809
1.1	0.605	0.473	0.782
1.15	0.635	0.482	0.759
1.2	0.671	0.512	0.763

Table 1: Relationship between Stimulus Voltage and Height Ratio of Peaks

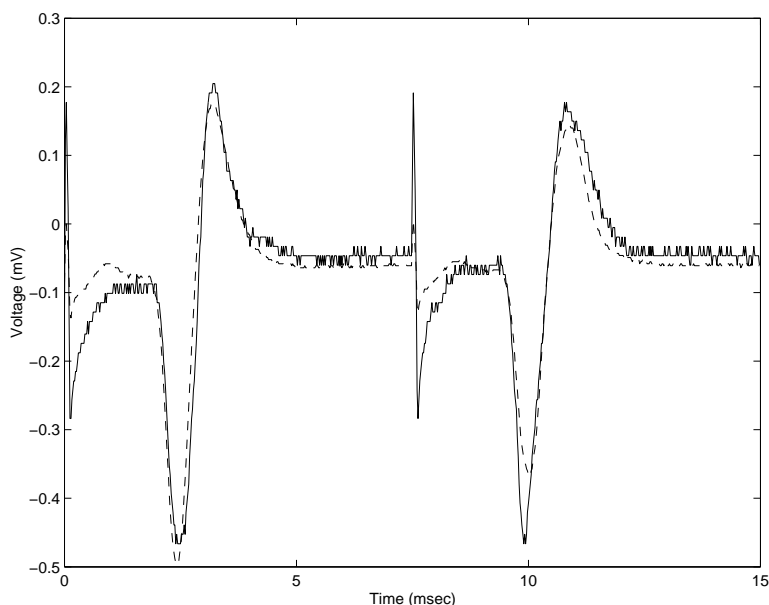


Figure 1: Normalized nerve responses to stimulus amplitudes of 1.2 mV (dashed line) and 0.9 mV (solid line).

amplitudes for the first pulse in both measurements are the same. However, the response due to the second pulse is much larger in the measurement of the smaller stimulus.

A summary of our results can be found in Table 1. These results indicate that as we decrease the stimulus amplitude, the response to the second pulse becomes more and more similar to the response for the first pulse. This relationship is plotted in Figure 2.

The last part of our experiment involved repeating measurements used to determine the refractory period as a control. Figures 3 and 4 show the results of this comparison. Both the response at the beginning of the experiment (solid line) and the response at the end of the experiment (dashed line) are shown on the same scale. Figure 3 shows two pulses of 1.1 mV separated by a delay of 8 ms while Figure 4 shows a delay of 4 ms. Note that despite our attempts, the control measurement differs significantly from the original measurement; the amplitudes of the second pair of stimulus pulses in Figure 3 are clearly different from each other.

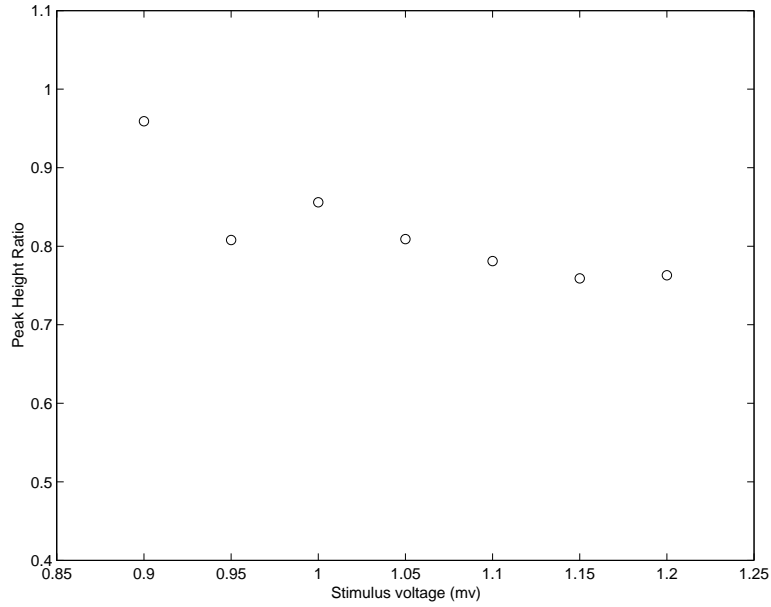


Figure 2: Height ratios for different stimulus amplitudes

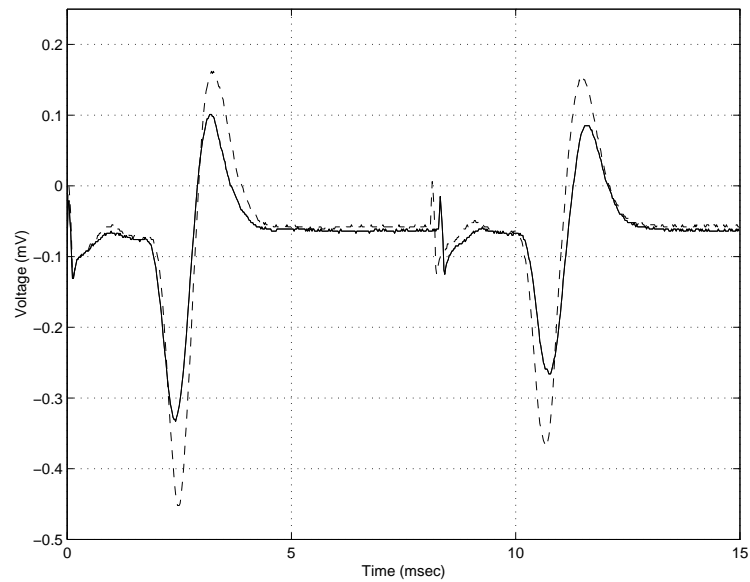


Figure 3: Original measurement (solid line) and control (dashed line) with 8 ms pulse delay

4 Discussion

To a limited degree, the results we obtained in the lab support our hypothesis. We had predicted that as the amplitude of a pair of stimulus pulses decreases, the refractory effect should diminish and eventually disappear. Our data supports that conclusion somewhat. If our hypothesis was correct, then the plot in Figure 2 should show a monotonically decreasing

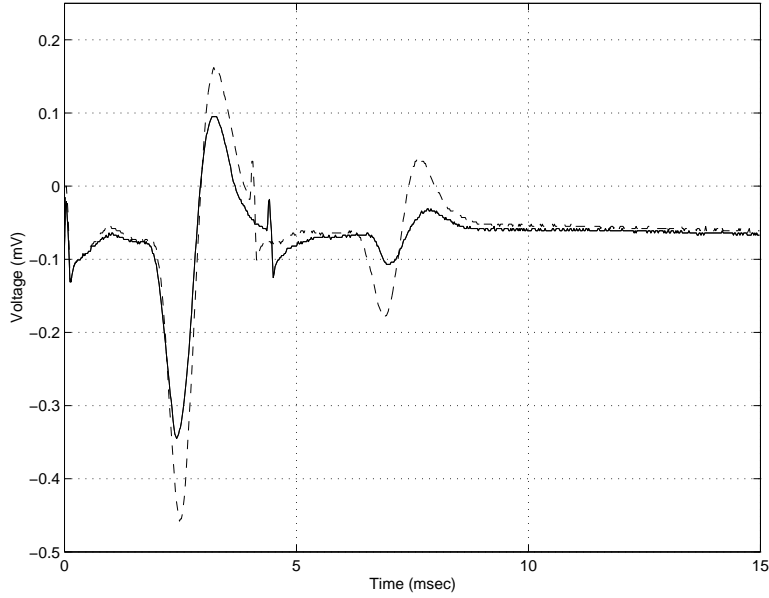


Figure 4: The measurement (solid line) and control (dashed line) with 4 ms pulse delay. Note how the second stimulus pulse interferes with the first CAP.

curve that approaches one for sufficiently small stimulus voltages. Except for the outlier where the stimulus voltage equals 0.95 mV, that is exactly what we see in Figure 2.

However, there are a number of problems. First of all, we have no explanation for the outlier in Figure 2. In addition, we had a number of problems conducting our experiment that resulted in unreliable data. During the course of our experiment, we inadvertently disabled the amplifier. Puzzled by the complete lack of signal, we tried a number of solutions. In our haste, we may have exposed the nerve to too much current, thereby damaging it. All measurements made after that point are suspect. For that reason, we do not consider any data points made after we believe the nerve was damaged.

One indication that something went wrong lies in Figures 3 and 4. The same measurement with the same stimuli taken at the beginning and end of the experiment produced the pair of curves in each figure. But the measurement taken at the end of the experiment shows a higher response than the first time. Ordinarily, we would expect that continued experimentation with the nerve would cause its performance to degrade over time, but here we see the opposite trend.

There are several possible explanations for this discrepancy between expectation and experiment. One is that our intuition is wrong and that repeated stimuli over the course of an hour enhanced neural responses. Another possibility is that the before and after trials were not really identical. For example, adding extra Ringer's solution towards the end of our testing period may have had some impact on the nerve. Alternatively, we might have misconfigured the signal generator and used a slightly different stimulus. The graphs in Figures 3 and 4 support that notion in that both the time delay between the two pulses and the magnitude of the stimuli are not exactly constant between the two before and after cases. Unfortunately, that sort of analysis poses a number of subtle challenges. Because we

are only seeing the response of the nerve to the input signals and not the actual stimuli, changes in neural behavior could alter the effective stimulus delay and magnitude.

Ultimately, we really do not know what the effect of our mishandling was on the nerve, so we have no choice but to discount all data that follows the handling point. We do have confidence in the data that came before, both because the experiment went well and because the data from that period is self consistent. We started out our experiments by looking at the response to a pair of 1.1 mV pulses separated by 8 ms. While examining the responses to different magnitude stimuli, we repeated this experiment. The results were almost identical. Because we had (inadvertently) placed a mini-control test in the middle of our experiment, we can now salvage part of our results even though the rest are uncertain.

Although our experiment has raised a number of new questions, we believe that those questions can be easily resolved by repeating our experiment while being more careful to maintain the health of the nerve at all times. In summary, our experimental work lends credence to our hypothesis, but is too uncertain to fully support it without additional experimentation.

5 Works Cited

[1] T. F. Weiss, *Cellular Biophysics*. Cambridge, MA: MIT Press, 1996.

[2] Department of Electrical Engineering and Computer Science, *The Compound Action Potential of the Frog Sciatic Nerve*. Cambridge, MA: MIT, 2001.

A Project Proposal

A.1 Hypothesis

Below a particular stimulus threshold, a pair of pulses separated in time with an interpulse delay of less than the refractory period should produce identical responses.

A.2 Background

The individual nerve fibers comprising the sciatic nerve have different thresholds for triggering Action Potentials. A stimulus pulse of low enough intensity would only trigger a small fraction of nerve fibers, resulting in a small CAP. Consequently, stimulus with a pulse of the same magnitude during the refractory period of the first pulse would not elicit any response from the fibers which generated the response for the first pulse. However, other fibers that did not respond to the first stimulus will respond to the second creating a CAP in response to stimulus pulses separated in time by less than the refractory period.

A.3 Methods

Before we begin, we will have completed that basic observations outlined in the lab manual. These observations include measuring the threshold voltage, identifying response components, and creating monophasic responses.

Initially, we will attempt to measure the refractory period by stimulating the nerve with pairs of high intensity pulses and trying to determine the minimum amount of time between pulses such that two CAPs result. That minimum delay is the refractory period.

Specifically, we will use pulses of 100 microsecond duration repeated at a rate of 10 per second with an amplitude of slightly more than the threshold voltage we measured in our basic observations (probably several tens of microamps). Our criteria for determining if a second CAP was actually generated is to verify that candidate second pulses follow the first CAP response by a time period approximately equal to the interpulse delay used for stimulant pulses. In addition, candidate pulses must be essentially identical in shape, amplitude, and width to the first CAP response in order to qualify.

Once we know the refractory period, we will stimulate the sciatic nerve with repeated pairs of pulses. Each pulse in a pair will be identical, and the two pulses will be separated by a period of time less than the refractory period measured above. We will conduct several measurements, each time reducing the intensity of both pulses until we determine the minimum intensity threshold needed for refractory effects to disappear.

As a control, we will quickly measure the refractory period after our experiment.

B Experimental Protocol

C Critiques