Action Potentials

Case study

You are working for a pharmaceutical company attempting to develop new treatments for sufferers of multiple sclerosis. In order to gain a better understanding of the condition you have been asked to design and conduct an experiment against which your treatments can be compared.

Hypothesis 1

Increasing stimulus strength will increase the peak of the compound action potential.

Prediction of results 1

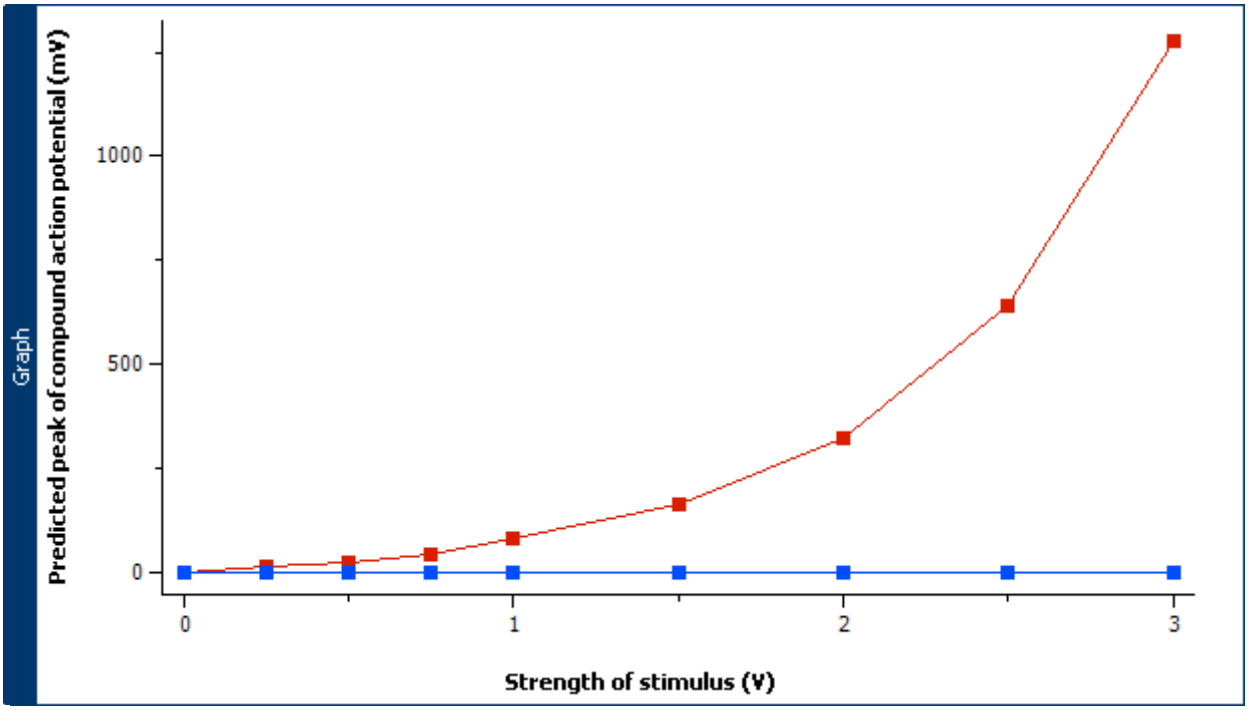

Figure 1: Prediction of results from increased stimulus strength on the Compound Action Potential peak on the sciatic nerve of the Bufo Marinus. The two plots represent theoretical data if the hypothesis is confirmed (red) and if the response was unaffected (blue).

Results 1

Table 2. Peak of compound action potential (mV)				
Strength of stimulus (V)	Replicate 1	Replicate 2	Replicate 3	Mean
0.00	0	0	0	0
0.25	0.069	0.055	0.051	0.0583
0.50	0.143	0.182	0.639	0.3213
0.75	3.493	4.564	5.469	4.5087
1.00	5.681	6.147	6.698	6.1753
1.50	7.858	7.186	7.5	7.5146
2.00	7.536	7.454	8.446	7.8120
2.50	8.018	7.916	8.592	8.1753
3.00	8.177	8.834	8.692	8.5677

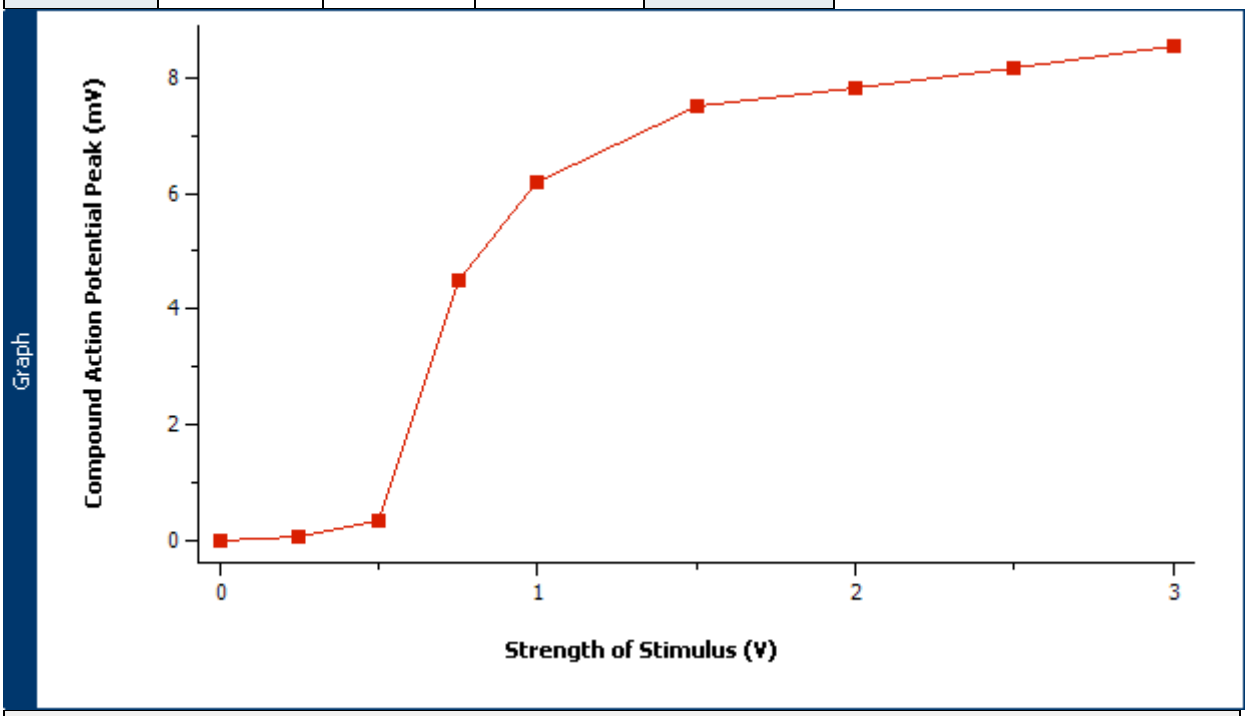

Figure 2:

The tested relationship between the Compound Action Potential peak (mV) and the strength of the stimulus (V). The graph represents the relationship of the mean values over three replicated experiments.

Comparative analysis

Table 3:

Comparision of Maximum Peak Compound Actional Potential (mV) and Latency period of the Compound Action Potential (msec) between the sciatic nerves of three different Bufo Marinus.

Group	Maximum peak compound action potential (mV)	Latency period of that compound action potential (msec)
Your own	8.56766	1.01
Alternate 1	18.864	1.01
Alternate 2	13.789	0.19

Hypothesis 2

When local anaesthetic is applied to the Bufo Marinus' sciatic nerve, it blocks the sodium channels by inhibiting the gates to go from inactive to closed, thus preventing the channels to become active again. Due to this behaviour, the longer the time period post exposure to the local anaesthetic, the lower the Compound Active Potential peak (mV) will be.

Materials & methods 2

In order to test the hypothesis, the following methods were followed to set up the lab and then conduct the experiment. To set up the lab, it was necessary to first attach the Power Lab recording and stimulating alligator clips to the correct locations. Once all of the connections were attached, and plugged into the Power Lab base, a diagnostic test was done to test run the electrical set up, using a piece of damp filter paper across the electrodes. Upon confirmation that the equipment was functioning correctly, the sciatic nerve needed to be dissected from a Bufo Marinus leg.

Once the lab was set up, the experiment commenced. The Lab Tutor was set to put off a stimulus of 0.75V twice, each stimulus within 5msec of eachother. Using a stopwatch, this stimulus was to be applied and recorded every thirty seconds after application of the local anaesthetic, Lignocaine 20 mg/mL. Once the computer was set up, a negative control was conducted in which two 0.75V stimuli were applied to the Bufo Marinus' sciatic nerve without any local anaesthetic. The results of this were recorded, and then four (4) drops of Lignocaine 20 mg/mL were applied to the sciatic nerve. Upon application, the stopwatch was started and the experiment was conducted every thirty seconds for four minutes. The results are summarised below.

Prediction of results 2

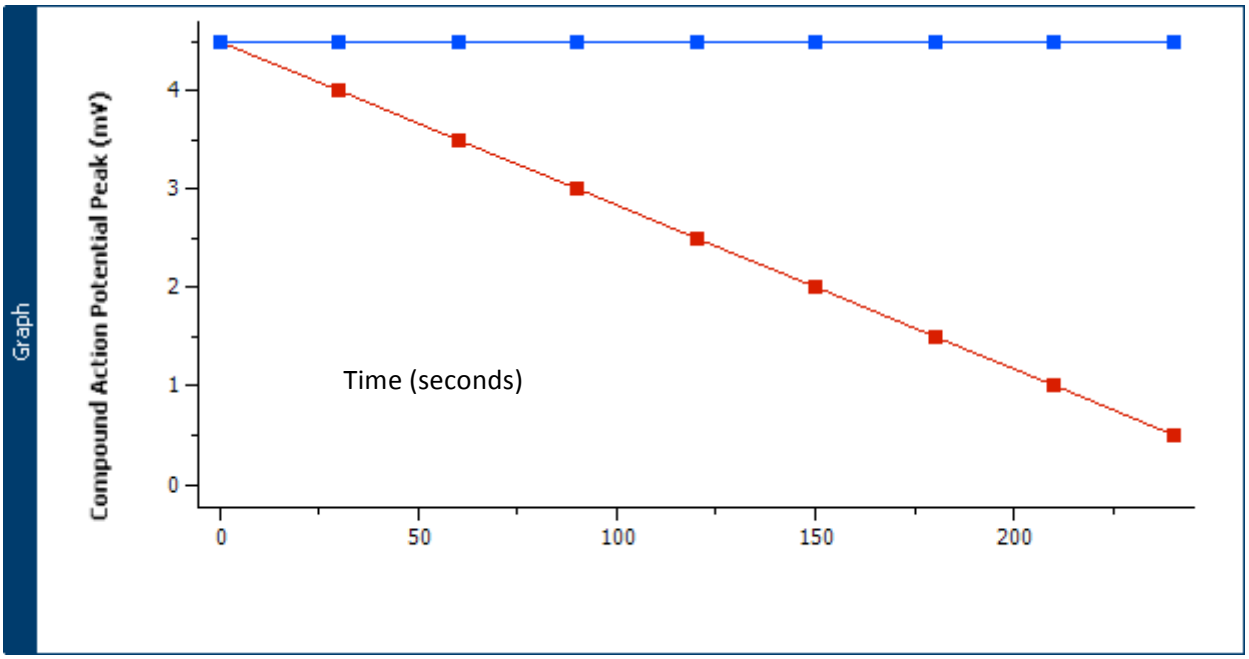

Figure 3.
The predicted results of the effects on the Compound Action Potential peak (mV) over time (seconds) elapsed since application of Lignocaine 20 mg/mL local anaesthetic. The red line predicts the results if the hypothesis is confirmed, and the blue line represents the results if there is no affect by the Lignocaine.

Results 2

Write a paragraph of text in the box below, describing the important trends and relationships for all data presented from experiment 2:

The raw data gathered in the experiment is summarised in Table 5 below. Due to the sciatic nerve dying after the first round of measurements, only one replication could be completed. However, the results from this replication confirmed the hypothesis that as time increased after applying the Lignocaine, the Compound Action Potential peak decreased. The first recorded result of 7.173 represents the negative control in which the experiment was run with no Lignocatine applied to the sciatic nerve. This result also helps provide a baseline to compare the rest of the experimental data too.

Figure 4 demonstrates the exponential decay resulting from our data. As time increased, the Compound Action Potential peak gradually approached zero. There was a sharp decrease within the first minute of application, however after two minutes, the graph starts to level off approaching zero.

Table 5.

Gathered Values of the Compound Action Potential Peak, from conducted experiment of applying Anesthetic (name) to the Sciatic Nerve of the Bufo Marinus

Independent Variable	Replicate 1	Mean
0.00	7.173	7.173
30.00	3.211	3.211
60.00	1.909	1.909
90.00	1.222	1.222
120.00	0.683	0.683
150.00	0.353	0.353
180.00	0.264	0.264
210.00	0.128	0.128
240.00	0.064	0.064

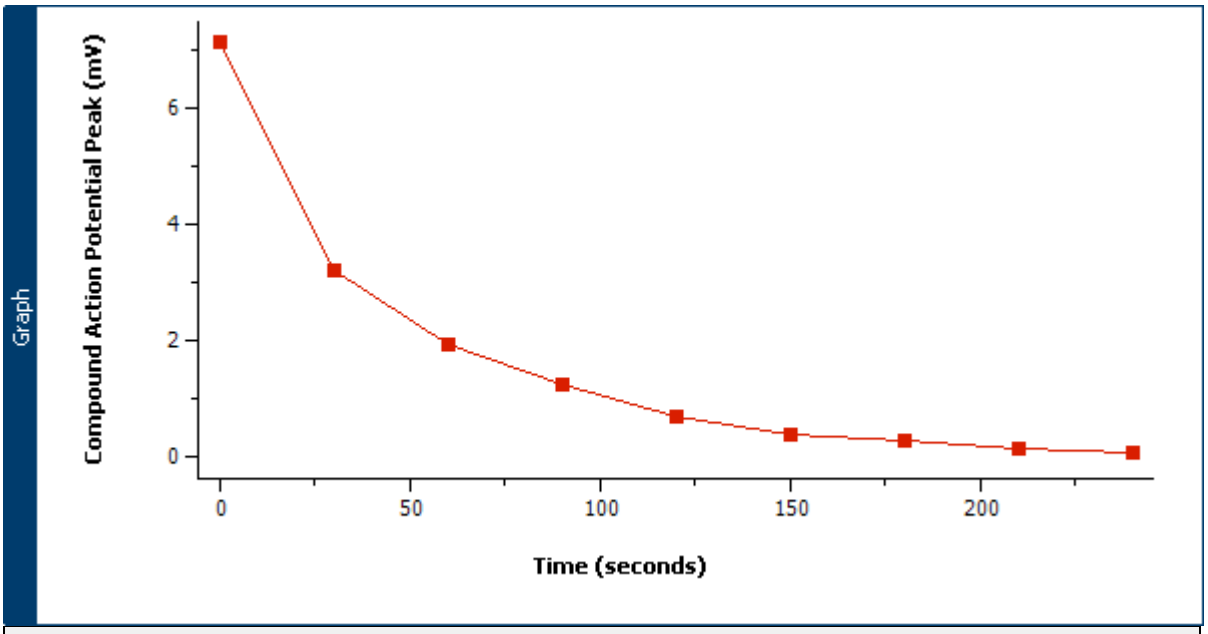

Figure 4.

Relationship between Time (second) since the Lignocaine 20 mg/mL was applied and the varying of the Compound Action Potential Peak (mV) value of the Bufo Marinus's Sciatic Nerve. The exponential decay of this graph confirms the experimental hypothesis.

Discussion

Remember to treat these questions like a short answer question in the final exam: be specific, clear, and concise.

1. Briefly describe (in complete sentences) whether the results of your first experiment confirm or disconfirm your hypothesis, and any errors in your data.

The results of our first experiment confirm our hypothesis that as the stimulus is increased, so too is the peak of the Compound Action Potential. There are potential errors in our data due to the reaction of the nerve after continuous stimulus. This was mitigated by applying Frog Ringer's solution between stimuli, however there may still be slight errors in our results.

2. Why do you think the peak of the compound action potential changes with different strength stimuli?

The peak of the compound action potential is the number of individual excited axons which pass under the first recording electrode. Thepeak of the compound action potential changed with the different strength stimuli because the stimuli strength were increasing. The compound action potential is the combined action potential of thousands of axons. As the different stimuli strengths are applied, different axons are excited due to the varying types of stimuli present in the Bufo Marinus sciatic nerve (Campbell, Reece & Meyer, 2009). As the stimuli strength was increased, so too was the number of axons excited, therefore causing an increase in the peak compound action potential.

3. What reasons can you give for the differences in peak and latency between nerves in your comparative analysis?

When experimenting on different individual nerves, it is expected that results will vary due to differing biological factors of the individual nerves to make each nerve unique. The comparative results showed large variances amongst the groups, especially in the peaks. This could have been due not only to differences in the actual nerves, but also differences in how the experiment was conducted. If a group stimulated the nerve with the same voltage several times in a row, there data could be compromised due to the nerve not responding as well over time. Another reason in the differences could have been the application of the Frog Ringer's solution. If it wasn't applied frequently enough, the nerve could have started to die, or died, causing the results to vary significantly.

4. Briefly describe (in complete sentences) whether the results of your second experiment confirm or disconfirm your hypothesis, and any errors in your data.

The results of Experiment two confirmed our hypothesis that as time increased, the compound action potential peak would decrease due to the application of a local anaesthetic, Lignocaine, to the sciatic nerve. This is evidenced by the exponential decay relationship seen on the graph in Figure 4. There were errors in our data however as the experiment was only conducted once. After the first replication was completed, and the Lignocaine was washed out of the nerve with Frog Ringer's solution, the nerve became unresponsive, preventing us from repeating the experiment. The lack of repetition reduces the strength of our results, however the first replication did in itself confirm our hypothesis.

5. What biological processes do you think have caused the trends your results illustrate?

Our results illustrated that as time increased after applying Lignocaine to the sciatic nerve, the peak compound action potential decreased. This is due to the effects of Lignocaine on the nerve. In cells, there are voltage-sensitive gated sodium ion channels. In a perfectly functioning cell, these ion channels work like a gated hinge. Upon stimulation, some of the active gates open, taking the channel from closed to slightly active. The Na^+ ions gradually begin to enter the cell, causing depolarisation. When a threshold level is met, the Na^+ ions enter the cell and generate a full action potential. At this stage, the gates are opened and there is a large influx of Na^+ into the cell. After the influx of Na^+, the active gate is closed, and an inactive gate opens. The inactive gate allows Na^+ ions into the channel, however they can not enter the cell. It is the process of the gates going from inactive to closed that allow the process to happen again, therefore this is a vital step in the activtion process (Campbell, Reece and Meyer, 2009).

When Lignocaine was applied to the sciatic nerve of the Bufo Marinus, this process of the Na^+ entering the cell was hindered. The Lignocaine prevented the sodium ion voltage sensitive gates to transition from the active to inactive to closed state. As the effects of the Lignocaine increased (as time increased), the more sodium channels that became stuck in the inactive state. This prevented the cycle to be able to repeat, therefore there was no influx of Na^+ ions into the cell. As fewer Na^+ ions entered the cell, the less individual axons which became excited as they weren't reaching the threshold level, and therefore didn't have an action potential.

6. In the case study you are working on multiple sclerosis, how do you think decreasing body temperature would affect patients with multiple sclerosis?

Decreasing body temperature could greatly help patients with multiple sclerosis. These patients suffer from the nerve cells being damaged and demyelinating. In a healthy person, myelination is the maturation of nerve cells. The layer of myelin acts as a layer of insulation around the cell, and allows the cells to travel faster and further due to the increased diameter of the cell (Campbell, Reece and Meyers, 2009). However, if the body temperature is decreased, then the action potential could have a longer duration. The longer the duration of the action potential, the higher the possibility that the nerves in someone who has multiple sclerosis would be able to communicate with one another and the nerve signal could be communicated and carried out appropriately (Edgar, Heller and Krilowicz, 1989).

7. You have a friend who has been diagnosed with multiple sclerosis who does not have a strong science background. Explain to them (using complete sentences) what causes their symptoms using language that they can understand.

The symptoms you are experiencing now are caused from the nerves in your brain and spinal cord being damaged. Usually, these nerves communicate with each other in order to move your arms and legs, or allow you to feel sensations on you arms and legs or even help you see. Since these nerves are damaged, they aren't able to communicate with the other nerves, and that is why you are experiencing these symptoms (Oksenberg and Stuve, 2010).

References

List any references you have used in the panel below:

Campbell, N. A. J, Reece, B., and Meyers, N., (2009). Biology. Frenchs Forest, NSW, Pearson Education Australia

Edgar, DM., Heller, HC., Krilowicz, BL. (1989). 'Action Potential Duration Increases as Body Temperature Decreases During Hibernation', *Brain Res.,* Vol 498, No 1, pp 73-80. Viewed 05 April, 2011, http://www.ncbi.nlm.nih.gov/pubmed/2790478

Oksenberg, J., Stuve, O., (2010). 'Multiple Sclerosis Overview', *PubMed*, Viewed 05 April, 2011, http://www.ncbi.nlm.nih.gov/books/NBK1316/?log$=disease8_quicklink#ms.Definition