Behaviour of the leopard frog (Rana sp.) sciatic nerve in response to increasing stimulation intensities

[redacted]

ABSTRACT

Throughout their natural lifespans, animals are exposed to a variety of environmental stimuli that differ both in kind and potency. An animal's ability to transduce these stimuli into signals that can be communicated throughout the body is essential to learning, reflexes, and other important components of animal behaviour. While it is commonly known that the action potential of individual neurons behaves in an all-or-none fashion when stimulated by threshold or suprathreshold stimuli, the fact that there may be billions of neurons contained within a single animal body (Moyes and Schulte 2016 p. 311) means that the behaviour of an individual neuron is relatively insignificant and unlikely to produce any noticeable change at a macroscopic scale. Thus, when analysing the physiology of muscle or organ systems, it can be more useful to consider how stimuli are transduced by larger groups of neurons, such as in nerves. In this study, we investigated the behaviour of the sciatic nerve of the leopard frog (*Rana* sp.) to determine whether its response was all-or-none or graded. We accomplished this by increasing the intensity of stimulus applied to the nerve and measuring the amplitude of resulting voltage changes at the exterior of the nerve. We found that the frog sciatic nerve exhibits a graded response. Increases in stimulation intensity above the threshold magnitude required for a response caused the magnitude of the nerve impulse to increase significantly above the magnitude observed at threshold (ex. 54.6±10.1% of maximum at 0.20V above threshold was significantly greater than the impulse recorded at threshold: 12.8±5.6% of maximum). In addition, we observed that when nerves were stimulated by intensities 0.40V or more above threshold, the nerve impulse magnitudes plateaued and did not change significantly (remaining between 87.6±7.2% of maximum and 97.8±4.4% of maximum). These observations are consistent with the explanation that the individual axons within nerves differ in their threshold potentials, and that increases in stimulation intensity causes increasing numbers of axons to be recruited until all the axons within the nerve have been recruited and the magnitude of the nerve impulse can no longer increase. Overall, our study provides support for our hypothesis that nerves exhibit graded responses in response to increasing stimulus intensities.

INTRODUCTION

Response of Individual Neurons to Electrical Stimulation

It has been well established that individual neurons communicate through a combination of graded and all-or-none responses. Graded responses are observed in the dendrites and cell bodies of neurons. When a dendrite is stimulated, this causes ion channels in the membrane to open, which in turn allows ions to cross the membrane, prompting a change in membrane potential that is dependent on the strength of the stimulus (Moyes and Schulte 2016 p. 162-163). This is a graded response, since stronger stimuli result in stronger responses. In contrast, what occurs at the axon hillock is characterised as an all-or-none response. At the axon hillock, integration of incoming graded potentials from the cell body and dendrites occurs. If the overall change in membrane potential is sufficient to reach or exceed the threshold depolarization for that neuron, an action potential will fire. If not, the signal decays and no action potential is triggered (Moyes and Schulte 2016 p. 164). This is an all-or-none response, since stimulation received by the neuron may result either in the generation of an action potential, or in no response at all.

Action potentials are propagated across long distances primarily through electrotonic conduction, which is an electrical phenomenon resulting from interactions between positive and negative charges at the membrane. However, while electrotonic conduction of action potentials is rapid, it is also imperfect: leakage of charged ions across the membrane and the resistance of the intracellular fluid cause voltage differences in membrane potential to decay over distance (Moyes and Schulte 2016 p. 191). Because electrotonic conduction is unable to transmit action potentials over long distances on its own, action potentials are regenerated periodically along the length of the axon by voltage-gated sodium channels (located at nodes of Ranvier in vertebrates). Thus, as long as action potentials do not decay below threshold before arriving at the next node of Ranvier, they are regenerated and continue to propagate down the axon. It is because of this that action potentials can be produced artificially by electrical stimulation, not only at the axon hillock, but also anywhere along the axon (Moyes and Schulte 2016 p. 173-174). This, too, is an all-or-none response. If the axon is stimulated with a threshold or suprathreshold depolarization, an action potential fires and propagates along the entire axon; otherwise, no action potential is formed.

The Nerve Response

But what happens when it is not a single axon that is stimulated, but an entire nerve, which is a collection of axons? In this study, we determine whether the response of a nerve to electrical stimulation is graded or all-or-none. By stimulating the sciatic nerves of leopard frogs (*Rana* sp.) with increasing stimulation intensities and recording the magnitude of the nerve impulses following stimulation at each intensity, we will be able to discern which pattern is followed by nerve impulses. We use the sciatic nerve in our study since it is one of the largest and longest nerves in the vertebrate body and therefore can be studied with relative ease.

We predict that the response will be graded. Individual neurons fire in an all-or-none fashion; however. if a nerve were also to exhibit an all-or-none response, this would require all of its associated axons to have identical threshold potentials in order to fire at the same time. This is highly unlikely, since an axon's threshold potential can be affected by a large number of factors, such as axon diameter (Zu and Terakawa, 2013), and the density and isoforms of

voltage-gated channels present in the membrane (Moyes and Schulte 2016 p. 184-185), all of which can differ between axons present in a single nerve.

MATERIALS AND METHODS

Subjects

For this experiment, ten adult leopard frogs (*Rana* sp.) of unknown sex that weighed between 30 and 60 grams were used. These frogs were killed through anesthesia, then dissected and their sciatic nerves extracted from the knee to the vertebral column. During dissection, tissues were bathed in Ringer's solution to keep them at physiological pH and ion concentration. The composition of Ringer's solution used in this experiment was 6.5g of NaCl, 0.14g of KCl, 0.15g of $CaCl_2 2H_2O$ and 0.2g of $NaHCO_3$ per litre of distilled water.

Experimental Design

Data were collected on January 10th, 11th, and 12th, of 2016 by students enrolled in the 2015W2 section of BIOL 363: Laboratory in Animal Physiology at the University of British Columbia from 3, 5, and 2 frogs on each of those dates respectively. The following procedure was performed for both sciatic nerves of each frog, yielding two data sets per frog (one per sciatic nerve).

Extracted nerves were moistened with Ringer's solution, then placed in nerve chambers which contained small volumes of Ringer's solution and were closed to the environment, and were therefore saturated by moisture. This was done to prevent nerve desiccation. Following protocol outlined in the BIOL 363 Lab Manual, recording electrodes were put in contact with the exterior of the nerve at two different locations along the length of the nerve, and connected to an oscilloscope whose trace served to visualize and record the magnitude of nerve impulses. Each nerve was stimulated at a rate of 30 to 40 times per second and the stimulation intensity increased gradually from 0.1V until either a plateau in the magnitude of nerve impulses was observed, or 1.5V stimulation intensity was reached, to minimize damage done to the nerve. For each stimulation intensity, the magnitude of the first deflection in the resulting biphasic response was recorded as a measure of the strength of the nerve impulse.

Data Analysis

For each frog, two data sets were obtained. However, only one data set from each frog (chosen at random) was used in analysis. This was done instead of averaging the data for each frog because, even on sciatic nerves extracted from the same frog, data may have differed considerably due to differences in laboratory technique. In particular, if one dissection took longer than the other, then the greater amount of time that had passed since the frog's death would likely contribute to a greater number of axons being non-responsive, decreasing the overall nerve response.

Additionally, data sets were omitted if they contained clear errors in lab technique or data reporting: for example, if they included oscilloscope readings that were more precise than that allowable by the equipment available, or if stimulus intensities were increased at increments greater than specified by protocol. This was done to ensure congruency between trials.

Two of the ten data sets used in this experiment are incomplete: although it appears that frog 1 from January 10th, and frog 5 from January 11th were both stimulated to the permissible

maximum of 1.5V, nerve impulse amplitudes were not recorded for all stimulations. For these data sets, I assumed that "missing data" represented amplitudes that did not differ from the most recently recorded amplitude, and completed the data sets accordingly.

Finally, as a result of differences in laboratory technique (as discussed previously) and biological variation, there was a large amount of variability in the data. Most notably, both the threshold stimulus intensity (the lowest stimulus intensity at which a non-zero nerve impulse was recorded) and the magnitude of the maximal nerve impulse varied substantially, ranging between 0.56-1.04V and 6-19mV respectively. I corrected for this variation by subtracting the threshold from each stimulus intensity, and by expressing the magnitude of nerve impulses as a percentage of the maximal nerve response. This normalization is justified since the objective of this study is not to determine specific values in describing the nerve response, but to make observations about its general shape, and these adjustments make it easier to compare the overall shapes of an average nerve response instead of comparing raw averages that are heavily influenced by irrelevant factors.

Statistical Analysis

For each stimulus intensity at or above threshold, the mean nerve impulse magnitude (as a % of the maximum nerve impulse attained for that nerve) and 95% confidence intervals were determined, then plotted. If confidence intervals overlapped in any of their values (including endpoints), this was taken as an indication that the means were not significantly different from each other. Conversely, if the confidence intervals did not overlap, the means were considered significantly different.

It is worth noting that although data for ten frogs was included in data analysis, the protocol used in this study meant that not all of the frogs were stimulated the same amount above threshold. For example, if threshold for a certain nerve was 1.02V, then it would only have been stimulated up to a maximum of 0.48V above its threshold. Thus, although the means closer to threshold represent sample sizes of n=10, sample sizes decrease gradually to n=5 as stimulation intensities increase far above threshold.

RESULTS

Description of Results

Below threshold stimulus intensity, no nerve impulse was observed.

As stimulus intensity surpassed threshold intensity, the nerve impulse increased gradually in magnitude. This trend is significant. For example, as shown in Figure 1, the magnitude of nerve impulse recorded at 0.20V above threshold (54.6±10.1% of maximum) was significantly greater than that recorded at threshold stimulus intensity (12.8±5.6% of maximum), and the impulse recorded at 0.80V above threshold (97.8±4.4% of maximum) was significantly greater than both of these.

After the initial increase, the amplitude of the nerve impulse plateaued as it approached its maximum response (Figure 1). For example, when the stimulus was increased by 0.40V or more above threshold intensity, none of the nerve impulses (ranging between 87.6±7.2% of maximum at 0.40V above threshold and 97.8±4.4% of maximum at 0.80V above threshold) differed significantly in magnitude from each other.

The stimulation intensity at which this plateau was reached varied depending on the replicate, ranging from 0.24V above threshold to 0.82V above threshold.

Figures

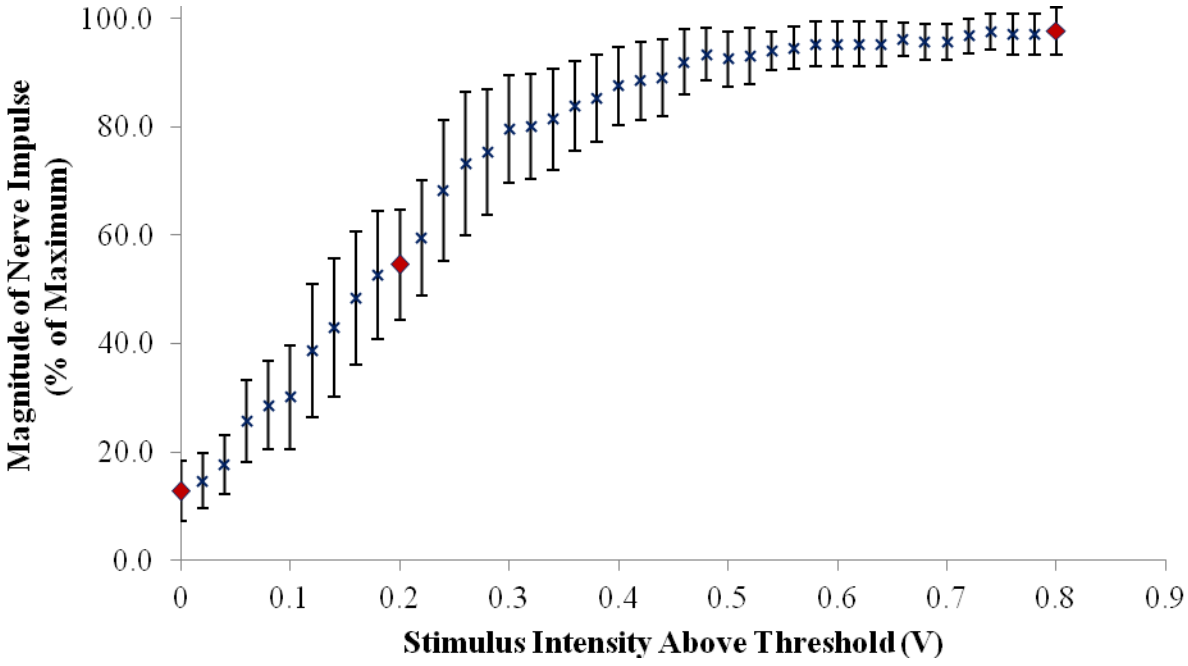

Figure 1. Effect of increasing stimulus intensity above threshold on the magnitude of the frog sciatic nerve impulse (mean ± 95% CI; ◆ indicates means significantly different (P<0.05) from each other; n=10 from 0-0.46V above threshold, decreasing to n=5 at 0.80V above threshold; sciatic nerves were extracted from the knee to vertebral column from adult *Rana* sp. of mixed sex weighing 30-60g)

DISCUSSION

A nerve impulse is the sum of many action potentials

In order to make specific predictions about the outcomes of this study, it is essential to understand how the nerve response was measured using our protocol.

The typical all-or-none action potential as characterized by the depolarization, repolarization, and after-hyperpolarization phases is observed in experiments such as those first performed by Hodgkin and Huxley (1952) on the squid giant axon, where the membrane potential within the axon is compared to the membrane potential without. However, it is often impractical to measure action potentials in this manner due to the small size of individual axons. Therefore, in this study, the recording electrodes were placed along the exterior of the nerve, so as to measure the sum of all action potentials within the nerve at once. If no axons reached threshold and no action potentials occurred, no voltage difference between the electrodes was observed. If action potentials were triggered in a small number of axons, a small voltage difference between the electrodes would be recorded. If stimulation was increased so that more action potentials were triggered within that nerve, a larger voltage difference would be recorded. Thus, the size of the measured nerve impulse is dependent on the number of action potentials being fired in response to a single stimulus.

Factors affecting the threshold potential of axons

Threshold potential is what determines whether an individual axon will fire an action potential. There are a number of ways in which threshold potential may differ between axons. For example, within a given nerve, axons vary in diameter (Johnson 2003, p. 96). Since axon diameter is inversely proportional to intracellular resistance (Moyes and Schulte 2016 p. 191), current flows more readily into a wider axon. Thus, axons with large diameters are more sensitive to stimulation, have lower threshold potentials, and are depolarized more effectively than their narrower counterparts when given the same stimulus (Xu and Terakawa 2013 p. 85). Axon threshold potentials may also be affected by the density of voltage-gated sodium channels in the membrane. Since these channels are required for initiating the depolarization phase of action potentials, any change in their density can have an effect on the excitability of the membrane to stimulation, and therefore on threshold potential (Moyes and Schulte 2016 p. 184-185). Finally, though less well understood, axons can differ in their sensitivity to stimulation depending on the isoforms of voltage-gated sodium or potassium channels present in their membranes (Moyes and Schulte 2016 p. 185).

The net result is that a single nerve is comprised of a large number of axons representing a wide range of threshold potentials. This lends itself to our hypothesis that nerves exhibit graded responses when subject to increasing stimulus intensity. If the nerve exhibits a graded response, we predict that as the stimulation of the frog sciatic nerve increases past the nerve's threshold potential, the magnitude of the nerve impulse will increase, and at some point will be significantly greater than the threshold response. The nerve's threshold potential occurs when the axon(s) with the lowest threshold potential in the nerve are stimulated. As the intensity of the stimulus is increased beyond this, more and more axons are recruited, and the sum of all action potentials propagating along the nerve past the recording electrodes, increases.

We additionally predict that as stimulation intensity continues to increase, a plateau will be reached whereupon further increases in stimulation intensity will not result in any significant increases in nerve impulse magnitude. At this point, stimulation intensity is sufficient to bring all (or nearly all) the axons within the nerve to threshold: this is the maximum nerve impulse, and increasing the stimulation intensity beyond this point will not continue to increase the magnitude of the impulse, since there are no more axons available to recruit.

My results aligned well with my predicted result. Compared to the threshold response, the nerve response at greater stimulation intensities was stronger. Since there were several points at which a suprathreshold stimulation resulted in a nerve impulse that was significantly greater in magnitude than threshold (see Figure 1), this indicated an increase of the nerve response with increasing stimulation intensity. In addition, a plateau was observed when the nerve was stimulated more than 0.40V above its threshold potential, as the magnitude of the nerve impulse did not vary significantly from this point even when stimulated with larger and larger stimuli.

Overall, this study provides evidence in support of my hypothesis that nerves exhibit a graded response when subject to increasing stimulus intensity. Unlike the all-or-none action potentials of individual axons, the magnitude of nerve impulses is proportional to the magnitude of stimulus presented. This provides a mechanism via which the strength of a stimulus can be encoded, which is essential in permitting a greater diversity of responses such as those seen in complex animal behaviour.

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