Recording of Action Potentials and Visualization of the Leech Ganglion using Intracellular Recording Techniques

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Abstract

Understanding the electrical properties, as well as the structures, of the cells in neural networks is a critical concept that neuroscientists must learn when it comes to studying the functions and dysfunctions of the brain. Neuroscience experiments has displayed that leeches can model current-dependent frequency research via the use of their Retzius cells located in their ganglion. We therefore decided to investigate how the leech cell responds electrically as different levels of current is injected. Using intracellular recording and an amplifier to send current into the Retzius cell, we measured the number of elicited action potentials to characterize the resulting action potential train. We found that increased current levels was observed to have a greater amount of evoked action potentials. These findings can provide insight into manipulating system sensitivity to treat patients with impaired senses as spike rates is associated with the receptive field intensity.

Introduction

To properly understand the function and structure of the brain, we must study the components that make up it. The brain relies on a cell's voltage change and the way cells communicates with other cells. Intracellular recording allows for the analysis of a cell's operations and anatomy. Additional technologies that work with intracellular recording, such as dye injection, allow us to map function to structure. Comprehension in this subject can give us leads in receptor and neurotransmitter function. Disorders pertaining to the dysfunction of these systems can now be worked on, now knowing where the problems may originate.

Leeches are a useful model for studying electrophysiological technologies as the durability of leech preparation makes it very accessible in educational facilities (Smith et al., 2004). Another important reason why leeches are good models is that they have Retzius cells which is a critical target when it comes to current injecting studies. They are big monopolar cells possessing a large axon that branches before leaving the ganglion. Spike rate, which is a metric from current injecting studies, is useful in providing information regarding different sense stimulus properties (Pirschel and Kretzberg, 2016).

Leeches are helpful when mapping basic sensory or motor fields to address the electrical characteristics of neural networks (Titlow et al., 2013). Its mechanosensory neurons are clearly defined and we can apply these concepts of receptive fields to senses other than touch, pain, and pressure (Pirschel and Kretzberg, 2016).

In this experiment, our purpose is to study how the leech Retzius cell responds to inputs of varying levels of injected current. We hypothesize that there will be more action potentials generated as a result of increased current. We will analyze the latency, spike count, and the inter-spike intervals of the action potential trains and compare it to the findings of a research paper. We will also be examining spontaneous action potential features and the procedure for filling the leech ganglia with fluorescent dye.

Methods

The BioAmp and PowerLab 26T was turned on and adjusted to the correct settings to prepare for recording. A petri dish with saline and the leech ganglion was put under the holder of the microscope. The ground wire was stuck into place with clay so that it is properly in the saline but at the edge of the dish. The microelectrode tip was prepared by filling it up with 3M KCl using a syringe, and then was placed appropriately into the holder. The manipulator knobs were used to lower the electrode tip into the saline to begin adjusting the noise, calculating the resistance, and balancing the bridge. The LabChart application was used to exhibit the noise, and appropriate measures of utilizing the ground clips were taken to reduce the noise as much as possible. Additional saline was added to the petri dish until it was full to reduce noise. LabChart was also used after initiating the Ohms Test, found on the amplifier, to calculate the resistance of the electrode. The resulting height of the square wave in volts is multiplied by 100 to give the electrode resistance in MΩ. The DC balance knob was then adjusted to show "rabbit ear" spikes to account for the resistance of the electrode.

The microscope, lights, and manipulator knobs were adjusted to bring the leech ganglia into focus while also having the microelectrode tip in view. Carefully, the electrode is moved closer to the Retzius cells as the magnification is increased all the way up to 11X. When prepared, the electrode was poked into the Retzius cell and LabChart began recording spontaneous action potentials. Within the recorded data, 10 trials of spontaneous action potentials were used to report specific metrics. The average resting membrane potential, or the voltage before the action potential, was measured in mV. The action potential height was analyzed by taking the absolute value of the action potential's peak to trough in mV. The spike width is measured by taking the width of the first peak at half the magnitude of the action potential height in seconds. The after-hyperpolarization amplitude is analyzed as the amplitude from resting membrane potential to trough in mV. The spontaneous firing rate was estimated by taking 10 different spike firing rates within 10 second windows in spikes per second.

LabChart's stimulator is set up so that a pulse will be sent with a 5 ms delay, a 0.5 Hz max repeat rate, 1 repeat, and a 500 ms duration. There are 4 current levels that are being tested for: -0.5 nA, 1.0 nA, 1.5 nA, and 2.0 nA. The pulse height parameter for the stimulator is converted and calculated as 1 nA of current is sent for each 10 mV. Current is then injected into the Retzius cell to observe on LabChart how the cell responds to the inputs. This is done with 10 trials per current level. The latency, or time between the beginning of the stimulus to the time of the first spike, is measured in ms. The first inter-spike interval is analyzed by finding the difference between the time of the first action potential and the time of the next in ms.

The metrics from each group are inputted on a shared class Google Spreadsheet to calculate and display the class cumulative data. Class data repeats were removed by taking the averages of them. A D'Agostino-Pearson test calculator from the web applet StatsKingdom was used to check for the normality of the metrics. If there are less than 10 data points, normality is assumed. Normally distributed data is reported with the mean and standard deviation. Skewed data is reported with the median and IQR. Raw data was uploaded from LabChart to Excel to make graphs. Figures such as histograms and scatterplots were made and analyzed on Excel.

A microelectrode tip is prepared with 5% Alexa Fluor 594 dextran dye. After, the pipette is filled with 3M KCl solution. The pipette is placed into the holder, and the previous procedure is repeated to check electrode resistance. 10 mV and 30 mV were calculated from 1 nA and 3 nA

for the required stimulator voltage. The double pulse setting for the stimulator was setup for a 1 second duration. The electrode was impaled into a neuron by checking for signals coming from the intracellular recordings. Red dye was injected into the cell by turning on passing pulses of current. After inspecting and waiting for this process to occur for 30 minutes, the leech ganglion was moved to a microscope slide to be viewed under a fluorescent microscope. A computer and Photoshop were used to upload and annotate the image of the resulting dyed cell.

Results

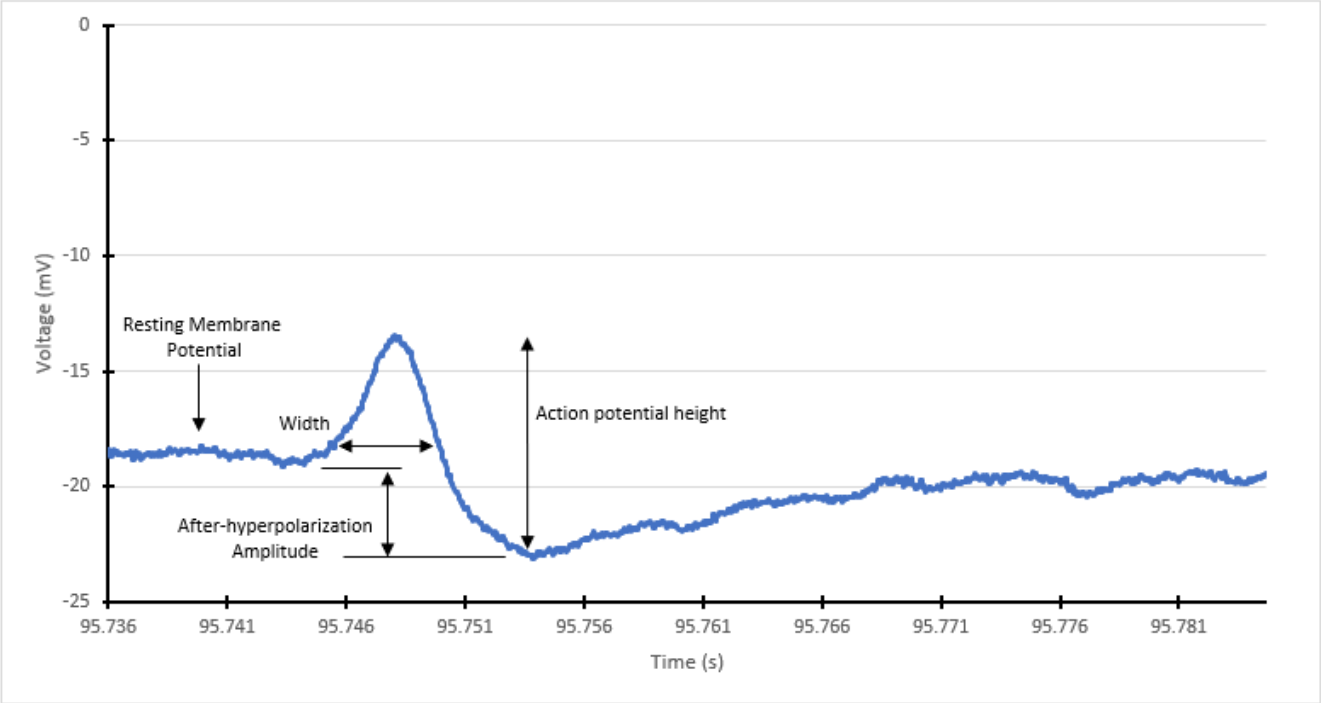

Figure 1. **Single spontaneous action potential firing of a leech Retzius cell, with metrics indicated.** Leech Retzius cell was impaled with a 29 MΩ resistance electrode to record spontaneous action potentials. The single-sided arrow represents the location of the resting membrane potential. The double-sided arrows denote the segments to derive spike width, action potential height, and after-hyperpolarization amplitude (AHP). The Y-axis shows the voltage recorded in millivolts and the X-axis shows the time recorded in seconds.

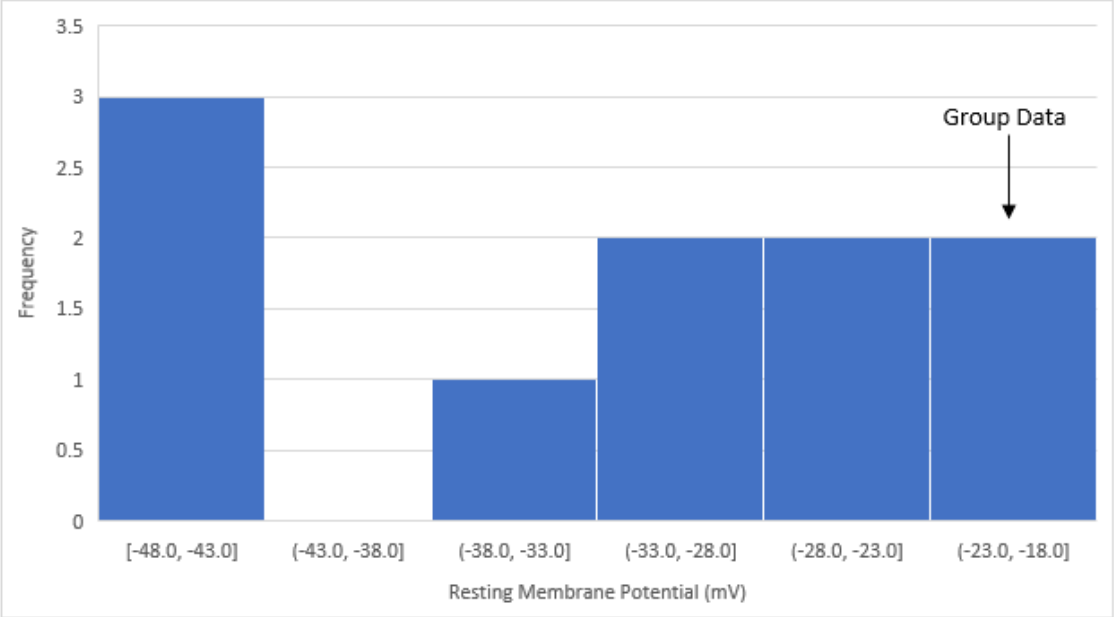

Figure 2. **Histogram of all class data of spontaneous action potential resting membrane potential, with group's data identified.** The resting membrane potential averages were calculated from 10 different spontaneous action potentials fired by the recorded cell. The values come from the class cumulative data. The Y-axis shows the frequency of how often each different values occur, and the X-axis shows the resting membrane potential recorded in millivolts. The arrow indicates the bin where the group data is located within the whole classes' data. $N = 10$.

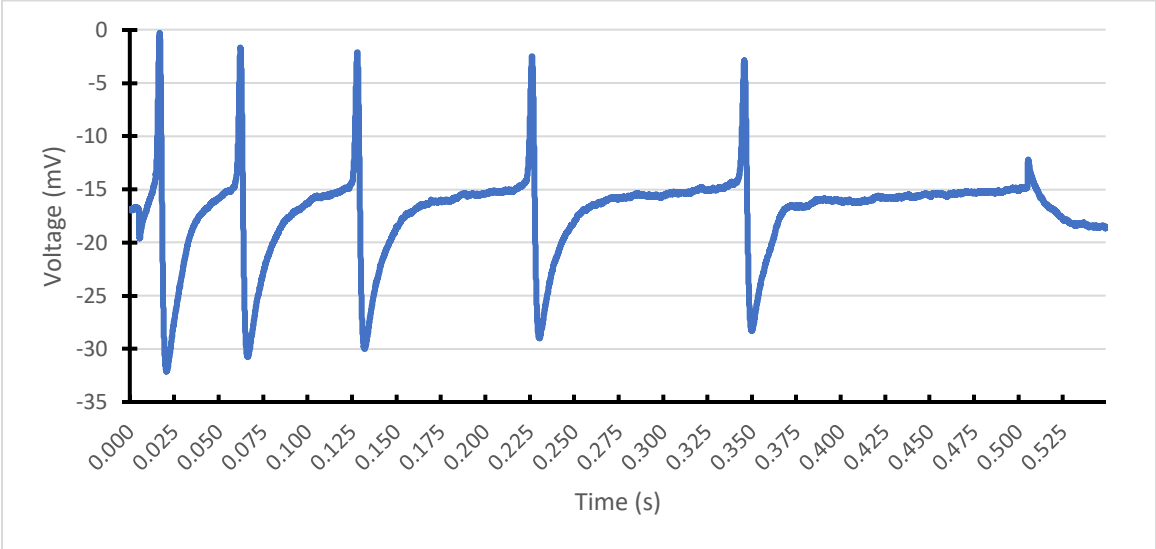

Figure 3. **Elicited action potentials of leech Retzius cell in response to 1 nA current.** 500 milliseconds injections of 1 nanoamp current was sent to stimulate the Retzius cell. The stimulator parameters consisted of a 5 ms delay, 0.5 Hz max repeat rate, 1 repeat, and 10 mV pulse height. The Y-axis shows the voltage recorded in millivolts, and the X-axis shows the time measured in seconds.

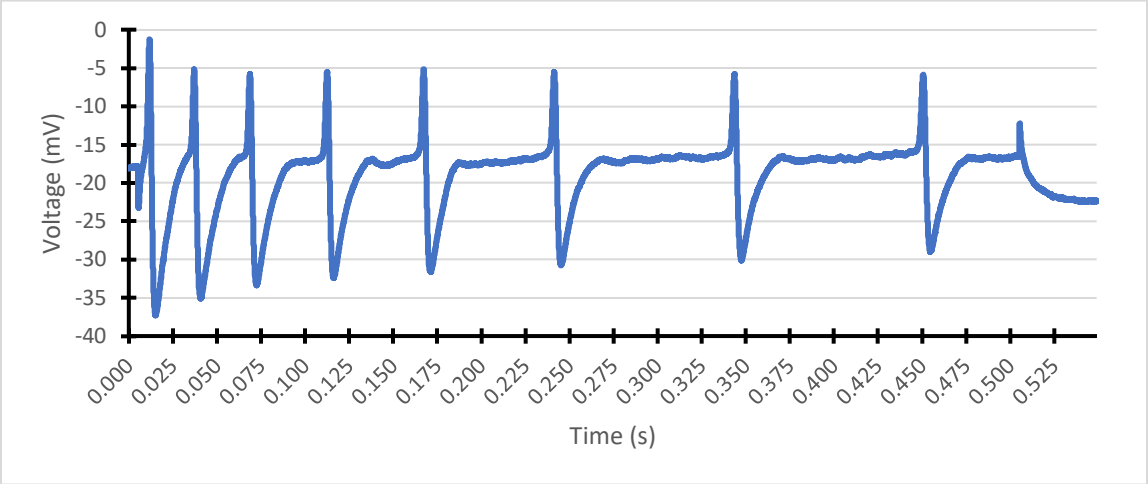

Figure 4. **Elicited action potentials of leech Retzius cell in response to 2 nA current.** 500 millisecond injections of 2 nanoamps current was sent to stimulate the Retzius cell. The stimulator parameters consisted of a 5 ms delay, 0.5 Hz max repeat rate, 1 repeat, and 20 mV pulse height. The Y-axis shows the voltage recorded in millivolts, and the X-axis shows the time measured in seconds.

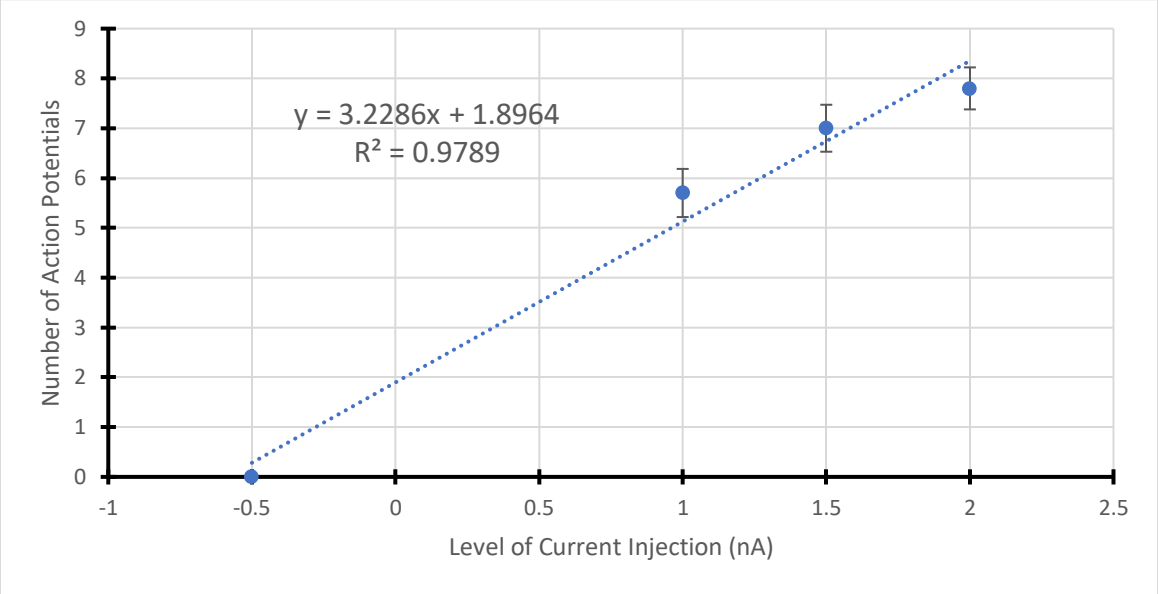

Figure 5. **Number of action potentials recorded for different levels of injected currents in the leech Retzius cell.** The data points are derived from taking the average number of action potentials from 10 trials for each level of 500 ms current injections. The blue line of best fit exhibits the linear equation $y = 3.2286x + 1.8964$ and its R^2 value is 0.9789. The Y-axis shows the number of action potentials recorded, and the X-axis denotes the level of current injection in nanoamps. In the plot, the error bars represent mean ± SD.

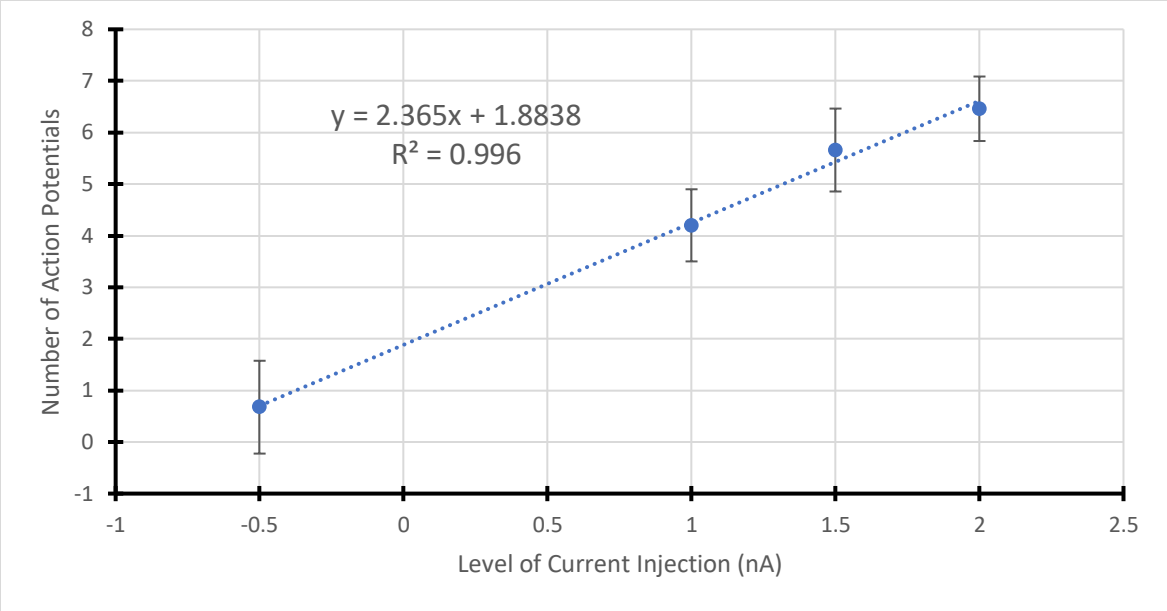

Figure 6. **Number of action potentials from class cumulative data for different levels of injected currents in the leech Retzius cell.** The data points are derived from taking the average number of action potentials from 8 class data means for each level of 500 ms current injections. The blue line of best fit exhibits the linear equation $y = 2.365x + 1.8838$ and its R^2 value is 0.996. The Y-axis shows the number of action potentials recorded, and the X-axis denotes the level of current injection in nanoamps. In the plot, the error bars represent mean ± SD.

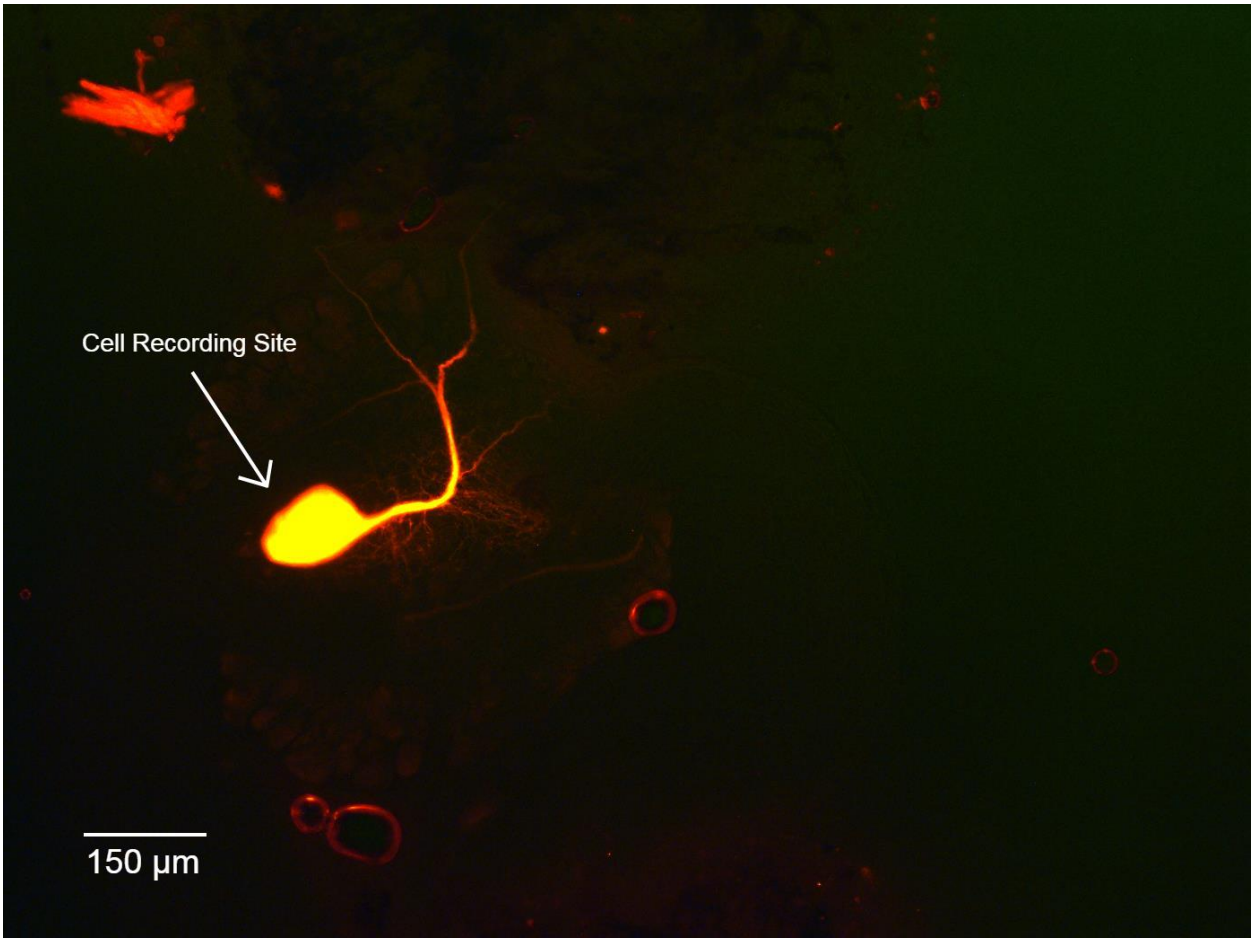

Figure 7. **Image of leech ganglion after dye filling.** An electrode that has Alexa Fluor 594 dextran dye in the tip was impaled into a neuron and injected into by passing pulses of current into the cell. The emission light from the dye is red. The arrow indicates which cell was recorded from. The scale bar reads 150 microns.

Data analyzed using the D'Agostino-Pearson Test reveals that 1 nA latency, 1 nA ISI, and spontaneous firing rate for the group data has a skewed distribution. Spike width from Section B02's data also has a skewed distribution. The following measured metrics for the single action potential and action potential train features are calculated from 10 trials or repeats. The resting membrane potential of the recorded Retzius cell is -22.0 ± 2.1 mV. The firing rate, or the estimate of the spontaneous firing rate of the cell, is reported to be 3.25 ± 0.25 spikes per second. The action potential height is 10.2 ± 1.7 mV. The spike width is reported to be $3.33e(-3) \pm 0.92e(-3)$ seconds. The after-hyperpolarization amplitude is reported to be 4.39 ± 0.47 mV. The following features are measured in response to a 500 ms duration, 1 nA current. The latency is reported to be 12.5 ± 2.16 ms. The inter-spike interval was reported to be 45.8 ± 4.4 ms.

The resting membrane potential of the cumulative class data is reported to be -32.6 ± 1.5 mV. The histogram of the class data appears to be slightly left-skewed (Figure 2), however the

D'Agostino-Pearson Test presents that the data is potentially symmetrically distributed. The group data appears on the right from the cumulative mean. The sample recording from Figure 4 appears to have 3 more action potentials compared to the recording in Figure 3. The trendline for Figure 5 and Figure 6 are consistent in that they showcase a positive, linear relationship.

Discussion

In this experiment, we used electrophysiological methods of intracellular recording to measure action potentials and inject dye into our targeted cells in order to gain a better mechanistic understanding of neuronal physiology at the cellular level. The technique allows for precision, which is important for studying the shape, electrical properties, and synaptic connections of specific target neurons in model organisms; therefore, the work can be translated into advancing humanity's progress in treating disorders related to neurotransmitters. With intracellular recording, it was observed that the resting membrane potential of the Retzius cell was reported to be -22.0 ± 2.1 mV. This finding was partially in agreement with what was found in the experiments done by Smith et al. (2004), as it was discussed that the membrane potential should be about -20 mV or lower. The standard deviation variability of our group's data falls into this statement for the most part, however, it is mentioned in the Smith et al. (2004) paper that a higher resting potential most likely indicates that the Retzius cell is unhealthy. Since our cell was borderline close, this may indicate that our cell was in near-poor condition which could've affected our other recorded metrics. The cumulative class data's average resting membrane potential of -32.6 ± 1.5 mV was even lower, which would be congruent with the findings.

Our experiments visually observed a decrease in inter-spike intervals and latency with increased level of current injection, as seen in Figure 3 and Figure 4. As also observed in Figure 5 and Figure 6, there is an increase in spike count with increasing levels of current injection. The trendlines for both graphs support a positive, linear relationship. This is consistent with the findings in the Pirschel and Kretzberg (2016) paper as they state that increases in spike counts usually correlate with decreases in latencies and inter-spike intervals. One important thing to note is that we only calculated the latency and inter-spike interval for the 1.0 nA level of current injection. Therefore, we cannot mathematically observe the decrease in inter-spike intervals and latency with increasing action potential count. A future experiment that we can look further into is analyzing the action potential train features into more depth. We haven't investigated adaption rate nor compared its relationship with injected current yet. Another thing that the Pirschel and Kretzberg (2016) paper does that we haven't done is experimenting with analyzing action potential train features of pressure, touch, and nociceptive cell bodies.

In our experiment, we used 5% Alexa Fluor 594 dextran dye which emits a red color. In the Titlow et al. (2013) paper, they used 3% Lucifer yellow dye which emits a yellow-green color. Our experiment's procedure was similar in that we also took 30 minutes for the dyes to diffuse. One thing that stood out to me was that the whole ganglia was diffused with green dye, however, our results shown in Figure 7 show that the dye mostly stayed concentrated within the injected cell and did not diffuse throughout. This brings up the question how the concentration or type of dye might affect the resulting image. We could also investigate the possibility of impaling

technique or just having leaky cells. We can conduct these experiments to develop efficient methods to achieve clear visualization of our target cells.

References

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