Extracellular Recording of Compound Action Potentials using the Giant Fiber System of Earthworms

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Abstract

The initiation and conduction of neuron action potentials are one of the few most important concepts that need to be understood in the field of neuroscience. The median giant fiber of the earthworm has demonstrated to be a functioning model to portray the properties and behaviors of action potentials. We therefore decided to investigate the effects of temperature on the conduction velocity of an earthworm's axon. Using intracellular recording and ice to manipulate temperature, we measured the medial giant fiber baseline action potential latency to determine conduction velocity at different temperature conditions. We found that the earthworm in lower temperatures had a significantly slower conduction velocity compared to when it was at room temperature. These findings can provide insight into how temperature can affect the conduction velocities of organisms other than earthworms and can also open to questions on how to prevent its effect.

Methods

A BioAmp and a PowerLab 26T was used to record. The recording needle electrodes were placed approximately one centimeter away from each other with a foam piece stuck in between them to prevent the alligator clips and wires from touching.

The earthworm was initially stimulated with 1 volt and the stimulus pulse height was gradually decreased until the compound action potential response was diminished. The max repeat rate and pulse width should be kept at 1 Hz and 0.2 millisecond, respectively. The stimulus that elicited an action potential 50% of the time was deemed the threshold. The sample recordings were visualized on LabChart to display the medial giant fiber compound action potential (MGF CAP) response. The pulse height from the threshold for the MGF is increased until an action potential for the lateral giant fiber (LGF) is elicited about 50% of the time. The pulse height was increased up to 3.3 V in an attempt to observe an action potential in the LGF.

The stimulus durations of the equipment were changed to 0.06, 0.08, 0.10, 0.20, 0.40, 0.60, and 1 millisecond where for each stimulus duration the stimulus amplitude was recorded in volts. The stimulus amplitude was computed using LabChart. The beginning of the MGF CAP response to its peak defined the amplitude. The stimulus duration and amplitude data were plotted on a scatterplot using Excel. A logarithmic trendline was used to form the strength-duration curve and to calculate the graph equation. The R^2 value also was given from the trendline. The rheobase was determined by using the experiment's lowest observed stimulus voltage data point. The trendline equation was used to calculate the chronaxie as it is represented by the stimulus duration at twice the rheobase.

The first recording pin was placed 2.5 centimeters away from the cathode stimulation pin. The same protocol to determine the threshold required to elicit an action potential from the earthworm's median giant fiber was repeated. The determined threshold stimulus voltage of 0.29 volts was administered to the earthworm axon for 5 trials. A handful of ice was then used to cover the worm. After 5 minutes, 0.29 volts was administered to the earthworm again for another

5 trials. The visualized data of the sample recordings on LabChart was used to determine the MGF baseline action potential latency in seconds. The time between the peak of the stimulus artifact to the peak of the MGF CAP response was defined as the latency. The conduction velocity in meters per second was then calculated by dividing the length of the first recording pin to the cathode stimulation pin (0.025 m) by the MGF baseline latency. Excel was used to graph a boxplot and to perform a statistical one sample t-test.

Results

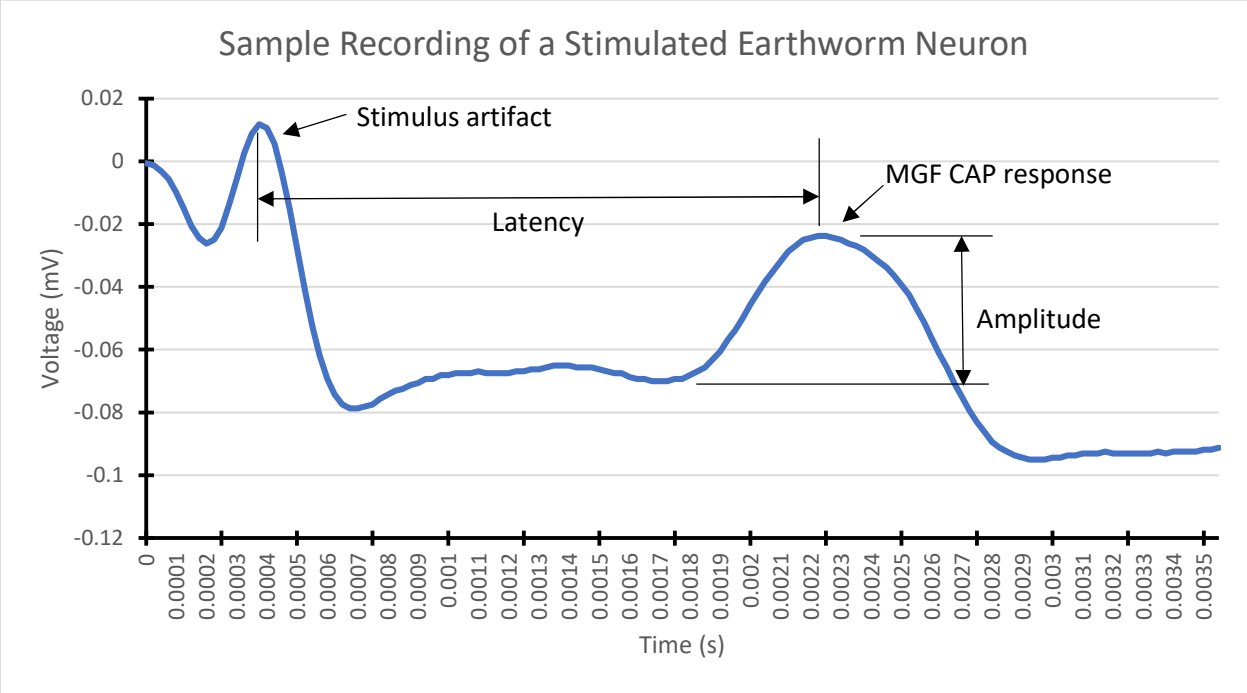

Figure 1. **Sample recording of an earthworm neuron that has been stimulated, with the stimulus artifact, medial giant fiber compound action potential (MGF CAP), latency, and amplitude marked**. Ventral nerve cord of the earthworm was stimulated with 0.23 V to elicit a MGF CAP response. The single sided arrows indicate the location of the stimulus artifact (first biphasic waveform) and MGF CAP response (second biphasic waveform) within the sample recording. The double-sided arrows denote the segments used to derive latency or amplitude of the CAP. The Y-axis shows the voltage recorded in millivolts and the X-axis shows the time recorded in seconds.

Table 1. **Compound action potential properties of the medial giant fiber indicating stimulus threshold, amplitude, and latency**. Characteristics of the CAP at the minimum stimulus voltage required to activate the MGF.

	Threshold	Amplitude	Latency
MGF	0.23 volts	0.046 millivolts	0.001925 seconds

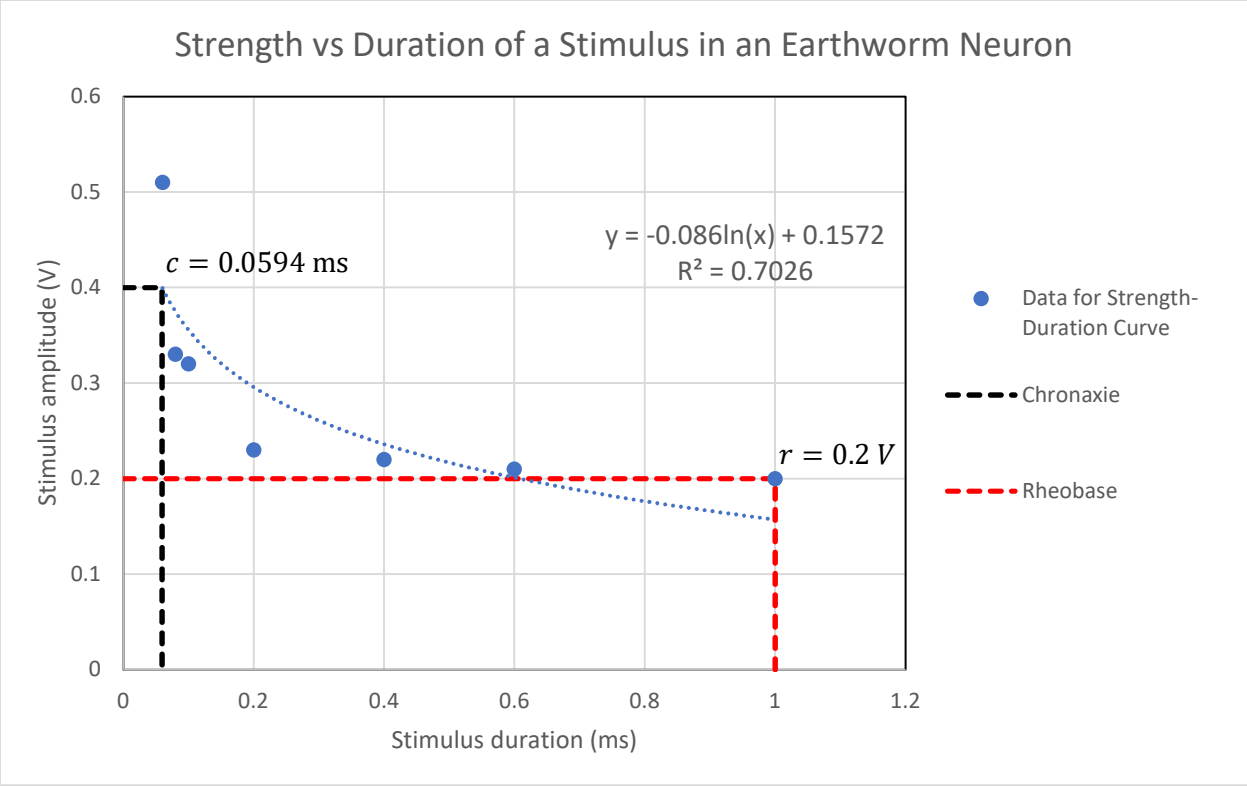

Figure 2. **Sample strength-duration curve for an earthworm with the rheobase and chronaxie identified**. Stimulus amplitude (in volts) was recorded at varying durations (in milliseconds) to calculate the rheobase and chronaxie of the earthworm medial giant fiber. Solid blue dots represents plots of data for the stimulus amplitude vs duration; black dashed line represents the axis values used to determine the chronaxie; red dashed line represents the axis values used to determine the rheobase; blue dotted line represents the trendline for the stimulus amplitude vs duration data. The trendline is exhibited as the logarithmic equation $y = -0.086\ln(x) + 0.1572$ and its R^2 value is 0.7026. The rheobase ($r = 0.2\ V$) is the minimum stimulus voltage that can elicit an action potential. The chronaxie ($c = 0.0594\ ms$) is the stimulus duration at twice the rheobase. N=7

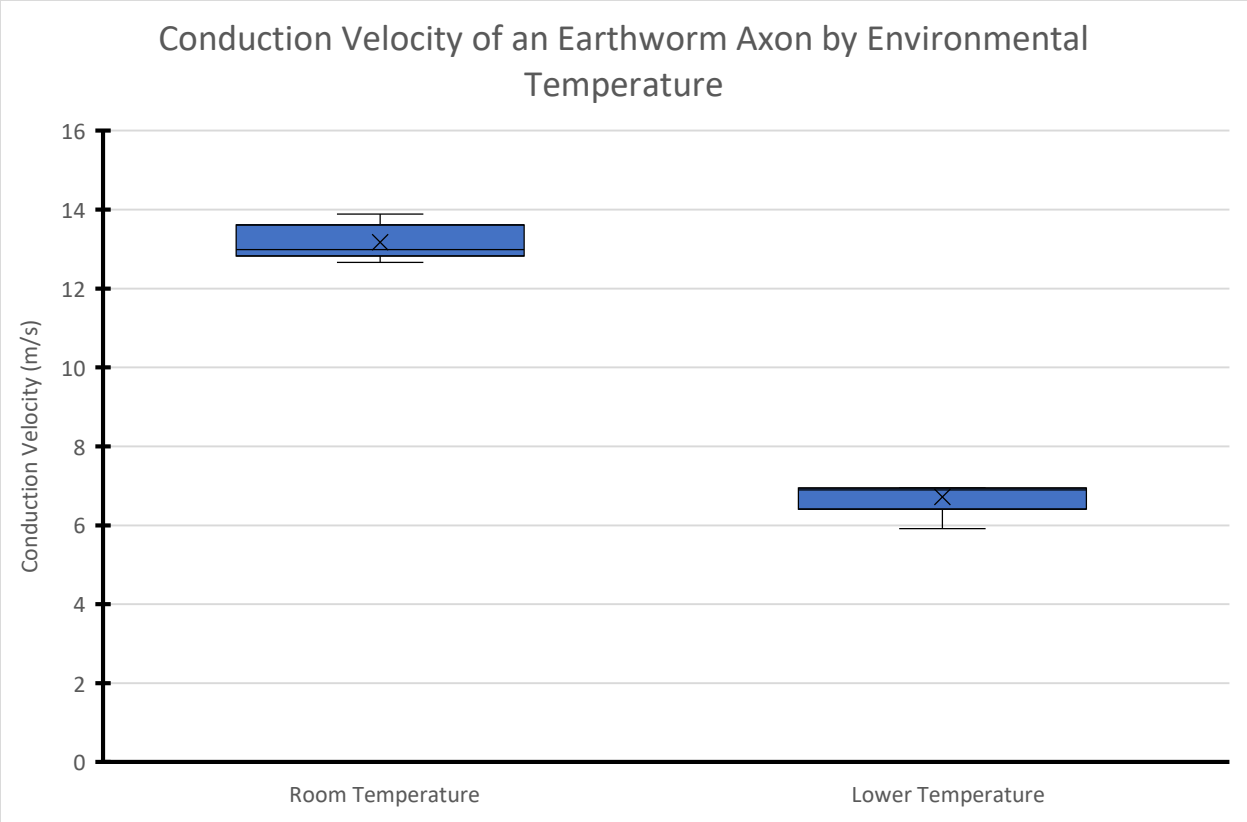

Figure 3. **Conduction velocity of an earthworm axon at room temperature and at a lower temperature**. Conduction velocity, in meters per seconds, was calculated after recording the MGF baseline action potential latency of the worm for 5 trials at room temperature and another 5 trials at a lower temperature. The mean of the room temperature box plot is 13.17 m/s and the mean of the lower temperature box plot is 6.72 m/s. The room temperature box plot is completely above the lower temperature box plot. There is a statistically significant difference between the conduction velocity of the room temperature worm and lower temperature worm as indicated by a p-value of 1.62×10^{-10} using a one-sample t-test.

Discussion

The experiment was performed in order to aid our understanding of how conducting extracellular recordings on earthworms can model various neuroscience concepts in the field. It was initially observed that the minimum stimulus voltage required to activate the MGF response was 0.24 V. Stimulus pulse height was increased up to 3.3 V with failure to observe an LGF CAP response. This result does not match to the experiments done by Kladt et al. (2010) as the paper displays success in achieving LGF threshold values. It is also mentioned in the paper that the MGF threshold is typically 1-4 V. However, it is important to note that their experiment used an electrical stimulus with a 0.5 millisecond duration, a longer duration compared to the 0.1 millisecond pulse width that was used. This brings up the question whether adjusting pulse width could aid in finding the LGF threshold. Alternatively, the earthworm maybe did not show the second response as the lateral giant fibers could just be damaged.

In the strength-duration curve experiment, it was concluded that the rheobase was 0.2 V and the chronaxie was 0.0594 ms. The value of the chronaxie was 0.060 ± 0.005 ms in Bähring et al. (2014) which is consistent. The differences in the Bähring et al. (2014) paper is that the rheobase and threshold stimulus at 0.1 ms stimulus duration is established as 1.012 ± 0.052 V and 1.49 ± 0.08 V, respectively. Given this information, a hypothesis could be made that the rheobase depends on the threshold stimulus more than the chronaxie does. More research can be done to find out why only the chronaxie value remains accurate when it is supposedly dependent on the rheobase and threshold stimulus.

A one-sample t-test for the conduction velocity experiment was performed to receive a T score of -31.48 and therefore resulted in a p-value of 1.62×10^{-10}. Since the p-value is less than 0.05, the null hypothesis that there should be no difference before and after the ice is applied is rejected. There is enough evidence to suggest that the conduction velocities of the room temperature worm and the lower temperature worm are statistically different. This is consistent with the Dierolf & McDonald (1969) paper's findings as it was stated that the cooling of the earthworms increased action potential latency which should then decrease conduction velocity.

Overall, it appears that the results from the papers shared a common trait of having a greater threshold stimulus than what was observed in the experiments. A plan of action for further research is to use multiple worms for accuracy as the Bähring et al. (2014) paper had a larger sample size of 74. Obtaining an accurate threshold may overwhelmingly be essential for making effective models. The Dierolf & McDonald (1969) paper also experimented on extracellular recordings of both cold- and hot-acclimated earthworms. The experiment has been conducted over 50 years ago and perhaps new findings can be made with present-day research equipment.

References

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