

The Conduction Velocity of Intact and Regenerated Earthworms

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Abstract:

Regenerative therapy is one of many techniques used to combat disease in neuroscience and cardiology. To improve regenerative therapeutics, it is necessary to better understand the mechanisms and physiological effects of natural blastema regeneration. Therefore, the conduction velocity of an earthworm animal model was studied to crudely understand the physiological effects of natural blastema regeneration. Specifically, electrophysiological recordings were generated on both intact and regenerated anesthetized earthworms via PicoScope and PicoLog. The conduction velocity of intact and regenerated earthworms was calculated as $18.9 \text{ m/s} \pm 9.0 \text{ m/s}$ and $15.5 \text{ m/s} \pm 6.2 \text{ m/s}$, respectively. These results indicate no significant difference can be drawn on intact and regenerated earthworm conduction velocities. It was also noted that the magnitude of action potentials increased following regeneration, which was not expected. Therefore, the mechanism resulting in a greater action potential magnitude will be examined in the future. The use of an effective procedure to investigate the electrophysiological properties of intact and regenerated earthworm gives potential to also investigate other earthworm electrical properties in the future.

Introduction:

Regenerative therapeutics is one of many techniques to combat disease in fields such as neuroscience and cardiology. However, translating this technique into effective clinical therapies has been inadequate to date. To improve regenerative therapeutics, it is necessary to better understand the mechanisms and physiological effects of natural blastema regeneration. To crudely understand the physiological effects of natural blastema regeneration, earthworm animal models can be used. In this study, the electrophysiological properties of intact and regenerated roundworms were examined. Specifically, the conduction velocity of intact and regenerated earthworm ventral nerve cord was analyzed.

Nerve conduction velocity is the measure of how fast a signal propagates across a nerve, thus loosely indicating how well the nerve acts in allowing information to travel.¹ It is often calculated by mechanically stimulating a nerve and measuring the time it takes the signal to travel from one end of the nerve to another.² Performing such an experiment on complex organisms such as humans is difficult, as various other signals are present and may interfere with the measurements. It is difficult to isolate the signal of a single nerve, so a fair amount of signal processing must be performed. For simpler organisms such as cockroaches, it is easier to gather a signal due to their size, but the presence of other nerves causes signal interference, resulting in indistinct signals. Earthworms avoid such difficulties, making them ideal in the study of nerve conduction velocity.² An earthworm contains a single, large, and myelinated, nerve cord that runs throughout the length of the worm (Figure 1).

Figure 1: Anatomy of an Earthworm³

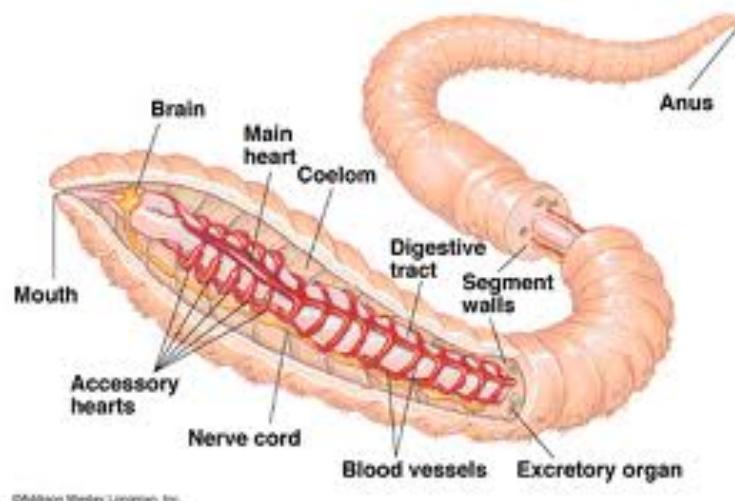


Figure 1: The anatomy of an earthworm is shown. An earthworm contains a single myelinated nerve cord that runs through the length of the worm.

The singular nerve cord reduces any significant nerve signal interference that may occur. Furthermore, the manageable size and slow-moving nature of an earthworm make it an ideal test subject.²

Earthworms, unlike humans and cockroaches, are capable of regenerating. In general, if up to a third of the posterior end of an earthworm is severed, it is capable of regenerating. As a result, earthworm studies give insight on more than just simple conduction velocity and nerve properties. The regenerative properties of earthworms give crude insight on regenerative electrophysiology. Analyzing regenerative electrophysiology in a simple organism such as an earthworm would create the baseline to build off of when considering the viability of regeneration. If the measured conduction velocity in a regenerated portion of a worm were equal to that of the original worm, there would be partial evidence supporting earthworm regeneration results in healthy, viable nerves. Applications of nerve regeneration are far reaching and affect thousands of people each year. If the mechanisms by which nerve tissue, even in worms, can be further

understood, the procedures through which neuroregeneration is initiated can be improved and translated to more complex organisms.

Procedure:

Preparation for PicoScope and PicoLog Analysis

To measure the conduction velocity of intact and regenerated earthworms, the programs PicoScope and PicoLog were used. A sample of worms were collected and kept alive in a large bin of soil. A single worm was taken out at a time and anesthetized in a prepared 10% ethanol solution for seven minutes. The anesthetized earthworm was pinned dorsal side up, and the leads were attached to the worm in the following manner (Figure 2).

Figure 2: Signal Capturing Schematic⁴

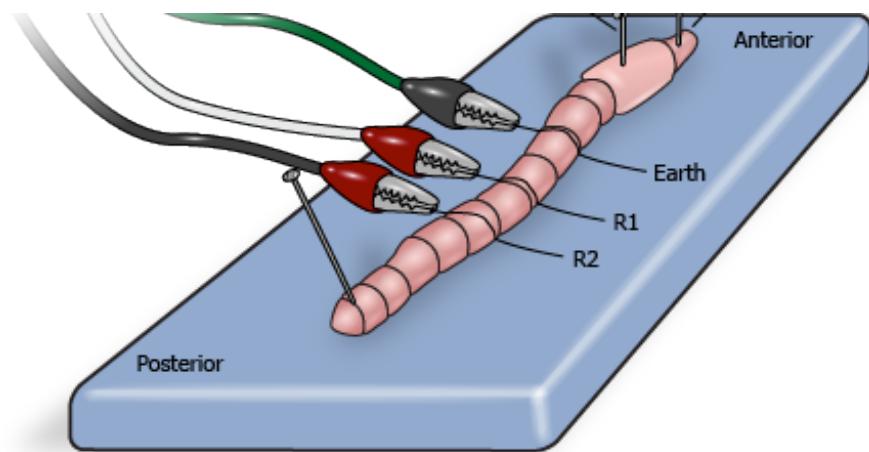


Figure 2: Two leads (R2 and R1) were placed two centimeters away from each other. A ground was placed anterior to R1.

The two leads, labeled as R2 and R1 in Figure 2, were placed approximately two centimeters away from each other. For individual signal measurements plotted on the same axis, each lead was attached to two different signal amplifiers. Both amplifiers were configured as follows (Table 1):

Table 1: Amplifier Settings

Input Select	Low Filter	High Filter	Mode	Gain
A	300 Hz	1 kHz	AC	100

The amplifier corresponding to R1 was connected to the “Channel A” input of PicoScope, while the amplifier corresponding to R2 was connected to the “Channel B” input. The ground was connected to the Faraday cage that the earthworm was placed in.

Spontaneous Measurements of Earthworms

Baseline recordings were taken for 2-second intervals with 200 milliseconds per division. These baseline recordings were made to distinguish between spontaneous and evoked measurements.

Evoked Measurements of Earthworms

Upon taking these baselines, the posterior end of the worm was mechanically stimulating with the end of a pen. Because two leads were plotted on the same axes, there was a slight offset in time between the peaks measured by the more posterior lead and the anterior lead. The data was analyzed in MATLAB to find the peaks that resulted from mechanical stimulation (see Appendix I for sample code). The time offset in peak location between leads A and B was measured and used to calculate the conduction velocity by:

$$\text{Conduction Velocity} = \frac{\text{Distance between leads}}{\text{Time}} \quad [1]$$

where the distance between the leads was set as 2 centimeters.

Regenerated Earthworms

Once these recordings were performed, the posterior third of the same worms were severed, and the worms were left to regenerate. The same worms were revisited three days later; unfortunately, only one of the two worms was fully regenerated. The same experimental procedure was followed to see if there was any significant difference in conduction velocity between the intact and regenerated nerve. The conduction

velocities were compared, even though no statistical test could result in a strong conclusion because of a small sample size.

Results and Discussion:

Spontaneous Measurements of Intact Earthworm

The intact earthworms naturally generated action potentials, making it necessary to distinguish between spontaneous and evoked measurements. Therefore, baseline recordings were taken for 2-second intervals with 200 milliseconds per division without any mechanical stimulation. These evoked measurements were produced only at the R2 lead. The spontaneous recording is shown below (Figure 3).

Figure 3: Spontaneous Recordings of Normal Earthworm

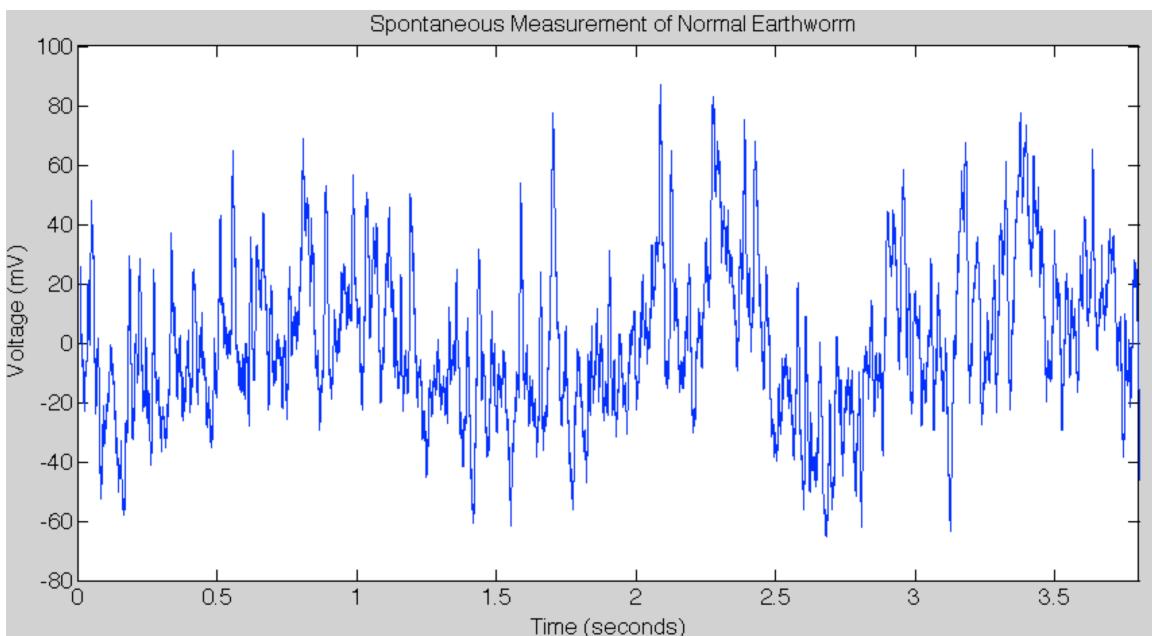


Figure 3: The baseline recordings were taken to distinguish between spontaneous and evoked measurements.

As expected, the spontaneous recordings of the intact earthworm had action potentials. None of these action potentials had a voltage of magnitude greater than 100 mV. Therefore, the threshold for a mechanically stimulated action potential was set to 100 mV.

Mechanical Stimulation of Intact Earthworm

After finding the threshold for mechanically stimulated action potentials, the trial was repeated where both leads were considered. The earthworm was poked numerous times with a pen, and the mechanically stimulated action potentials were recorded (Figure 4).

Figure 4: Mechanically Stimulated Action Potentials

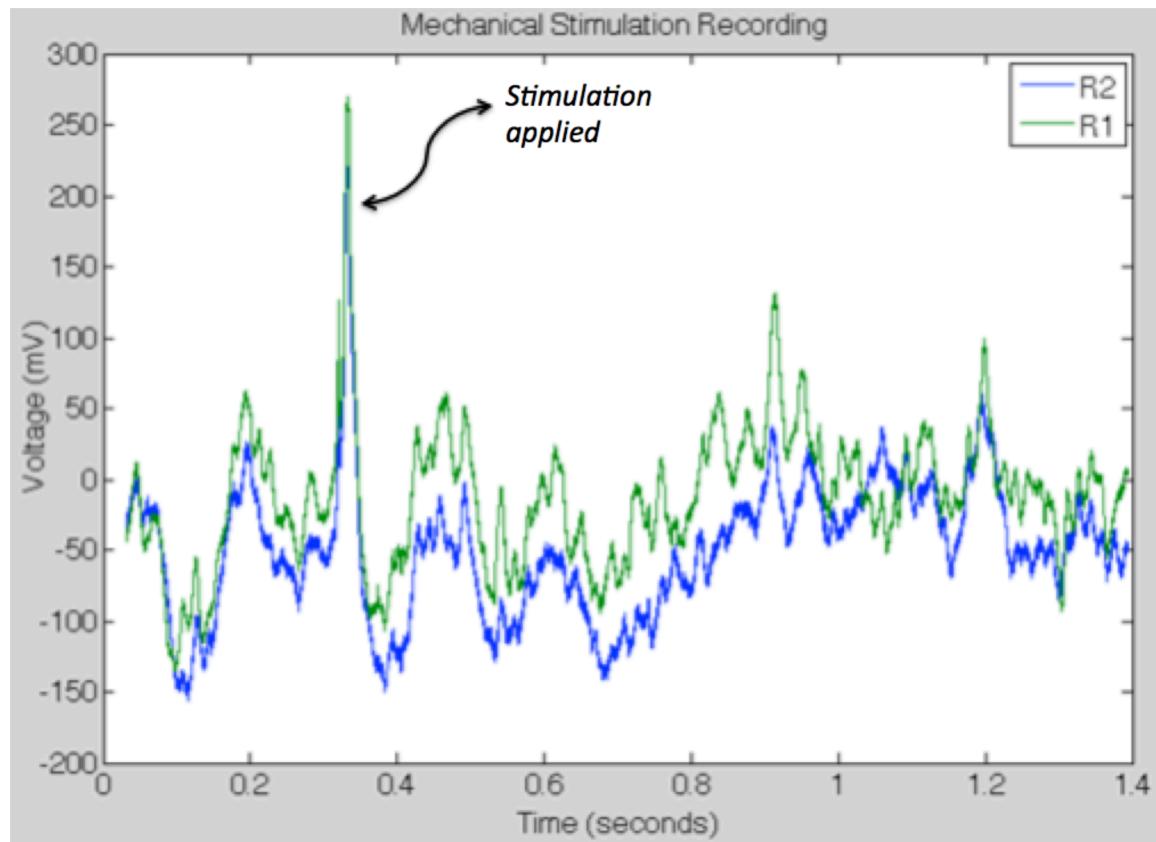


Figure 4: The recording of mechanically stimulated action potentials, where lead R2 is in blue, and lead R1 is in green. When the stimulation was applied, an action potential greater than 200 mV was apparent.

As previously indicated, the mechanically stimulated action potentials would have a magnitude greater than 100 mV. Specifically, they ranged from approximately 200 mV to 400 mV. These mechanically stimulated action potentials resulted almost instantaneously after poking the earthworm with the pen. From these action potentials, data analysis techniques were applied to zoom in on the mechanically stimulated action

potentials for each lead, as shown below (Figure 5). This was necessary to determine the conduction velocity, as the action potentials occurred so close to each other.

Figure 5: Identifying the Mechanically Stimulated Action Potentials

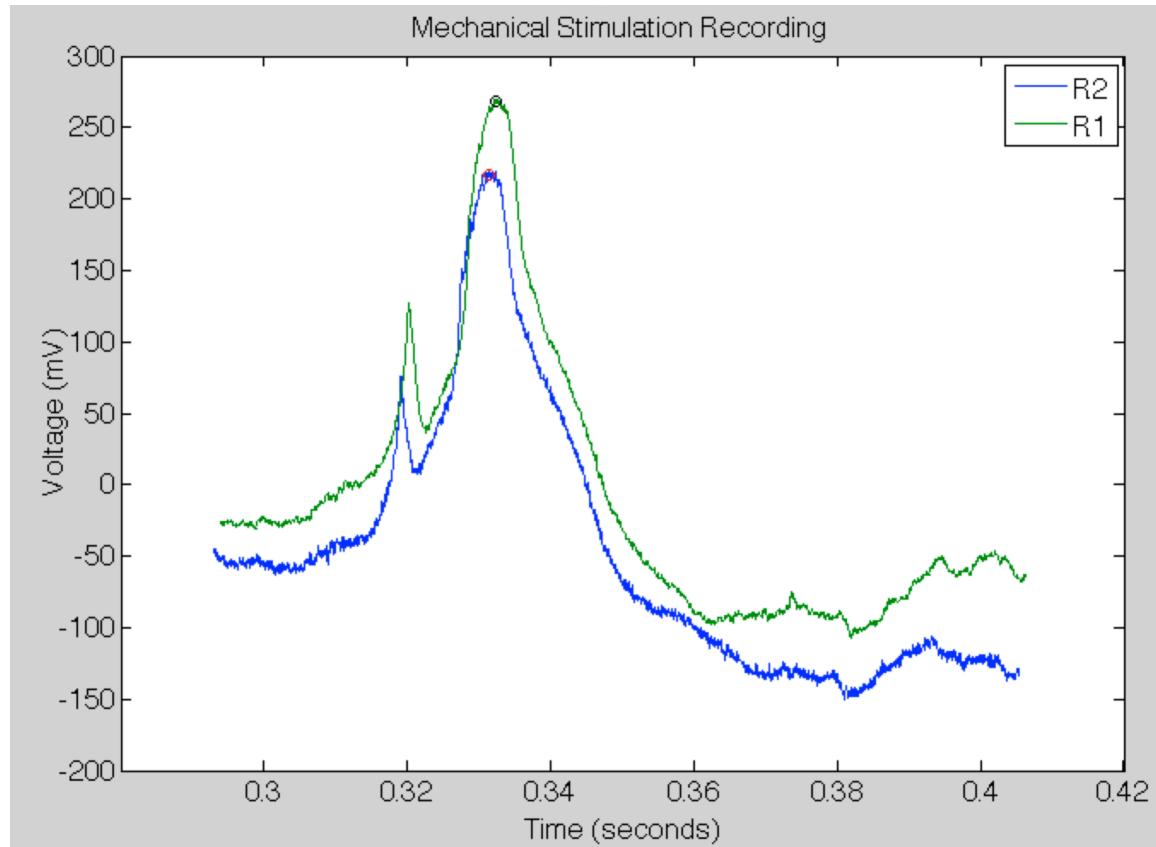


Figure 5: The mechanically stimulated action potential from Figure 4 was zoomed in on. The peaks of the action potentials for both leads were circled, as shown in the figure above. This allows for the conduction velocity to be calculated.

The circles in red and black from Figure 5 indicate the position of where the action potential peak was for leads R2 and R1, respectively. As expected, the action potential for lead R2 occurred before the action potential for lead R1 because lead R2 was in the more posterior position. From these circles, the time at which these action potentials were generated for each lead was determined. This process was repeated six times, as shown in Table 2.

Table 2: Time of Action Potential for Each Lead

Trial	R2 Peak Time (seconds)	R1 Peak Time (seconds)
1	0.3314	0.3324
2	0.9992	1.0008
3	2.2826	2.2832
4	2.8223	2.8231
5	4.7801	4.7816
6	6.6399	6.6420

As a result, the conduction velocity from each trial was determined by recalling that the leads were 2 centimeters apart (Table 3).

Table 3: Conduction Velocity for Each Trial

Trial	Conduction Velocity (m/s)
1	19.8
2	12.4
3	33.4
4	24.4
5	13.3
6	9.5

From Table 3, the conduction velocity of the intact earthworm was calculated as $18.9 \text{ m/s} \pm 9.0 \text{ m/s}$. In order to have an unbiased control group, the data was only generated for the single earthworm that regenerated after three days. Both earthworms were severed in the posterior third, in hopes to have more samples. However, as indicated previously, only one of the two earthworms regenerated after three days. This conduction velocity was compared to the conduction velocity of the regenerated earthworm for insight on electrophysiological differences between intact and regenerated earthworms. If more time were available, more earthworms would have been studied for greater statistical significance.

Spontaneous Measurements of Regenerated Earthworm

The regenerated earthworm naturally generated action potentials, making it necessary to distinguish between spontaneous and evoked measurements. Therefore, baseline recordings were taken for 2-second intervals with 200 milliseconds per division without any mechanical stimulation. The spontaneous recording is shown below (Figure 6).

Figure 6: Spontaneous Recordings of Regenerated Earthworm

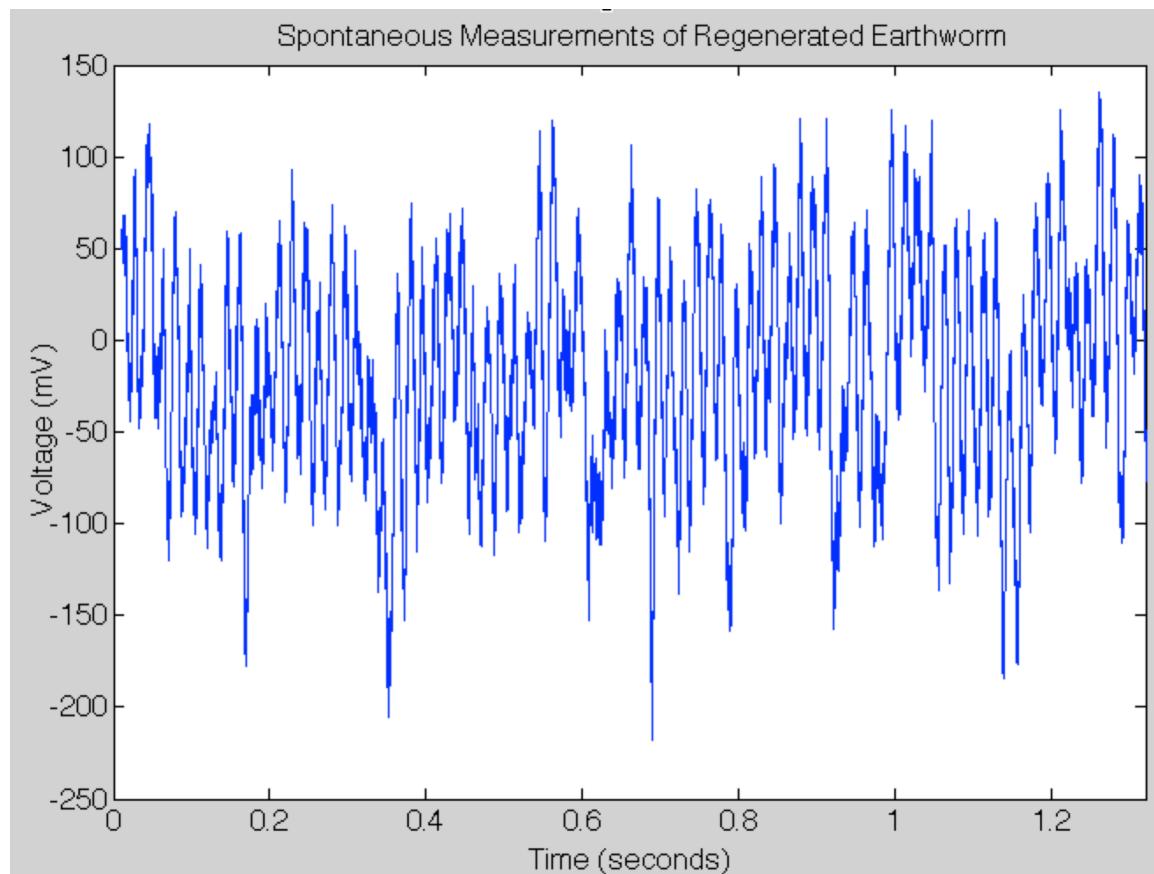


Figure 6: The baseline recordings were taken to distinguish between spontaneous and evoked measurements.

As expected, the spontaneous recordings of the regenerated earthworm had action potentials. Interestingly, these action potentials were of greater magnitude than the action potentials of the intact earthworm. Interestingly, many action potentials were greater than

100 mV for the regenerated earthworm, unlike the intact earthworm. This corresponded with higher mechanically stimulated action potential magnitudes, as shown below.

Mechanical Stimulation of Regenerated Earthworm

After finding the threshold for mechanically stimulated action potentials, the trial was repeated where both leads were considered. The earthworm was poked numerous times with a pen, and the mechanically stimulated action potentials were recorded (Figure 7).

Figure 7: Mechanically Stimulated Action Potentials

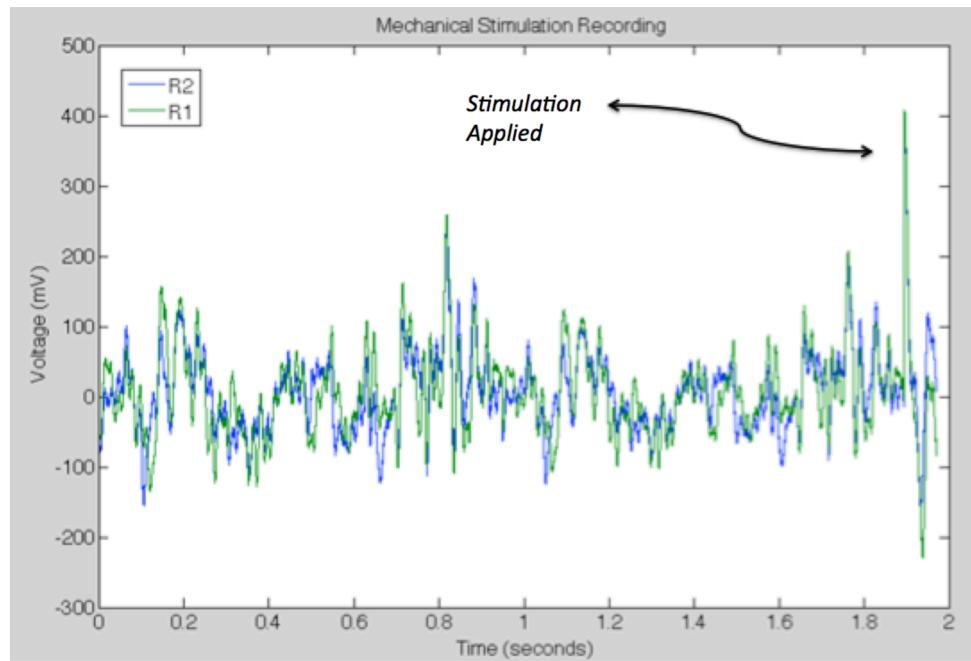


Figure 7: The recording of mechanically stimulated action potentials, where lead R2 is in blue, and lead R1 is in green. When the stimulation was applied, an action potential greater than 200 mV was apparent.

As previously indicated, the mechanically stimulated action potentials had a magnitude greater than 200 mV. Specifically, they ranged from approximately 300 mV to 500 mV, which was greater than the mechanically stimulated action potentials of the intact earthworms. This was not expected, as it was initially postulated that regeneration

of tissue should result in greater internal resistance, and thus a lower magnitude conduction velocity. This can be seen by examining the spatial decay length (λ) equation:

$$\lambda = \sqrt{\frac{r_m}{r_i}} \quad [2]$$

As the internal resistance r_i increases, the spatial decay length decreases, implying the conduction velocity should decrease. Future work might include examining what regenerative properties might result in a greater action potential magnitude following mechanical stimulation.

These mechanically stimulated action potentials resulted almost immediately after poking the earthworm with the pen. From these action potentials, data analysis techniques were applied to zoom in on the mechanically stimulated action potentials for each lead, as shown below (Figure 8). This was necessary to determine the conduction velocity, as the action potentials occurred so close to each other.

Figure 8: Identifying the Mechanically Stimulated Action Potentials

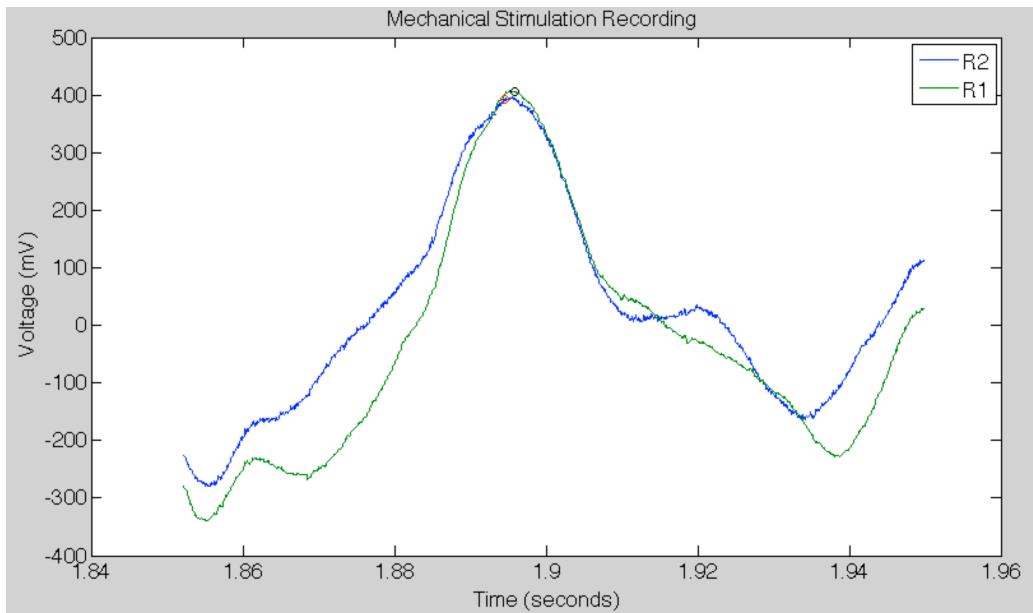


Figure 8: The mechanically stimulated action potential from Figure 7 was zoomed in on. The peaks of the action potentials for both leads were circled, as shown in the figure above. This allows for the conduction velocity to be calculated.

The circles in red and black indicate the position of where the action potential peak was for leads R2 and R1, respectively. As expected, the action potential for lead R2 occurred before the action potential for lead R1 because lead R2 was in a more posterior position. From these circles, the time at which these action potentials were generated for each lead was determined. This process was repeated three times, as shown in Table 4.

Table 4: Time of Action Potential for Each Lead

Trial	R2 Peak Time (seconds)	R1 Peak Time (seconds)
1	1.8945	1.8959
2	4.3643	4.3652
3	6.2222	6.2242

As a result, the conduction velocity from each trial was determined by recalling that the leads were 2 centimeters apart (Table 5).

Table 5: Conduction Velocity for Each Trial

Trial	Conduction Velocity (m/s)
1	14.3
2	22.2
3	10.0

From Table 5, the conduction velocity of regenerated earthworm was calculated as $15.5 \text{ m/s} \pm 6.2 \text{ m/s}$. While the conduction velocity of the regenerated earthworm was slightly less than the conduction velocity of the intact earthworm, no definitive conclusion can be drawn, as the results are both within one standard deviation of each other.

Due to time constraint, only a limited amount of electrophysiological investigation was possible. Furthermore, only small sample sizes were available, resulting in weak conclusions being drawn. Future work includes investigating the shape, duration, threshold, refractor period, spatial dimensions, temperature dependence, and

propagation of a larger sample size of normal and regenerated earthworm ventral nerve cord.

Conclusion:

To improve regenerative therapeutics, it is necessary to better understand the mechanisms and physiological effects of natural blastema regeneration. Therefore, the conduction velocity of earthworm animal models was studied to crudely understand the physiological effects of natural blastema regeneration. The conduction velocity of intact and regenerated earthworms was calculated as $18.9 \text{ m/s} \pm 9.0 \text{ m/s}$ and $15.5 \text{ m/s} \pm 6.2 \text{ m/s}$, respectively. These results indicate no significant difference can be drawn on intact and regenerated earthworm conduction velocities. However, it was noted that the magnitude of action potentials increased following regeneration, which was not expected. Therefore, the mechanism resulting in a greater action potential magnitude will be examined in the future. Future work also includes investigating the shape, duration, threshold, refractor period, spatial dimensions, temperature dependence, and propagation of a larger sample size of normal and regenerated earthworm ventral nerve cord.

References:

- ¹ Kandel, Eric R., James H. Schwartz, and Thomas M. Jessell. *Principles of Neural Science*. New York: McGraw-Hill, Health Professions Division, 2000. Print.
- ² Kladt, Nikolay, Ulrike Hanslik, Hans-Georg Heinzel. *Teaching Basic Neurophysiology Using Intact Earthworms*. The Journal of Undergraduate Neuroscience Education, 2010.
- ³ "Earthworms A Scientific Study | Complete Guide for Earthworms." *HubPages*. N.p., n.d. Web. 17 May 2013. <<http://sukritha.hubpages.com/hub/EarthwormAfraidSalts>>.
- ⁴ "Earthworm Action Potentials." *ADInstruments*. N.p., n.d. Web. 17 May 2013. <<http://www.adinstruments.com/solutions/education/ltxp/earthworm-action-potentials>>.

Appendix I:

Sample Code To Calculate Conduction Velocity

```
clear all;
csvRange1 = [9800,0,(12053),0];
x = csvread('Mechanical Stimulation A-B_09.csv',9800,0,csvRange1);

csvRange2 = [9800,1,(12053),1];
m = csvread('Mechanical Stimulation A-B_09.csv',9800,1,csvRange2);

csvRange3 = [9800,2,(12053),2];
k = csvread('Mechanical Stimulation A-B_09.csv',9800,2,csvRange3);

plot(x,m);
hold all;
plot(x,k);
[pks,locs] = findpeaks(m,'MINPEAKHEIGHT',205);
hold all;
xxx = locs(13);
plot(x(xxx),pks(13),'o');
[pks2,locs2] = findpeaks(k,'MINPEAKHEIGHT',200);
hold all;
xx = locs2(32);
plot(x(xx),pks2(32),'o');
diff = abs(x(xx)-x(xxx));
vel=.02/diff;
```