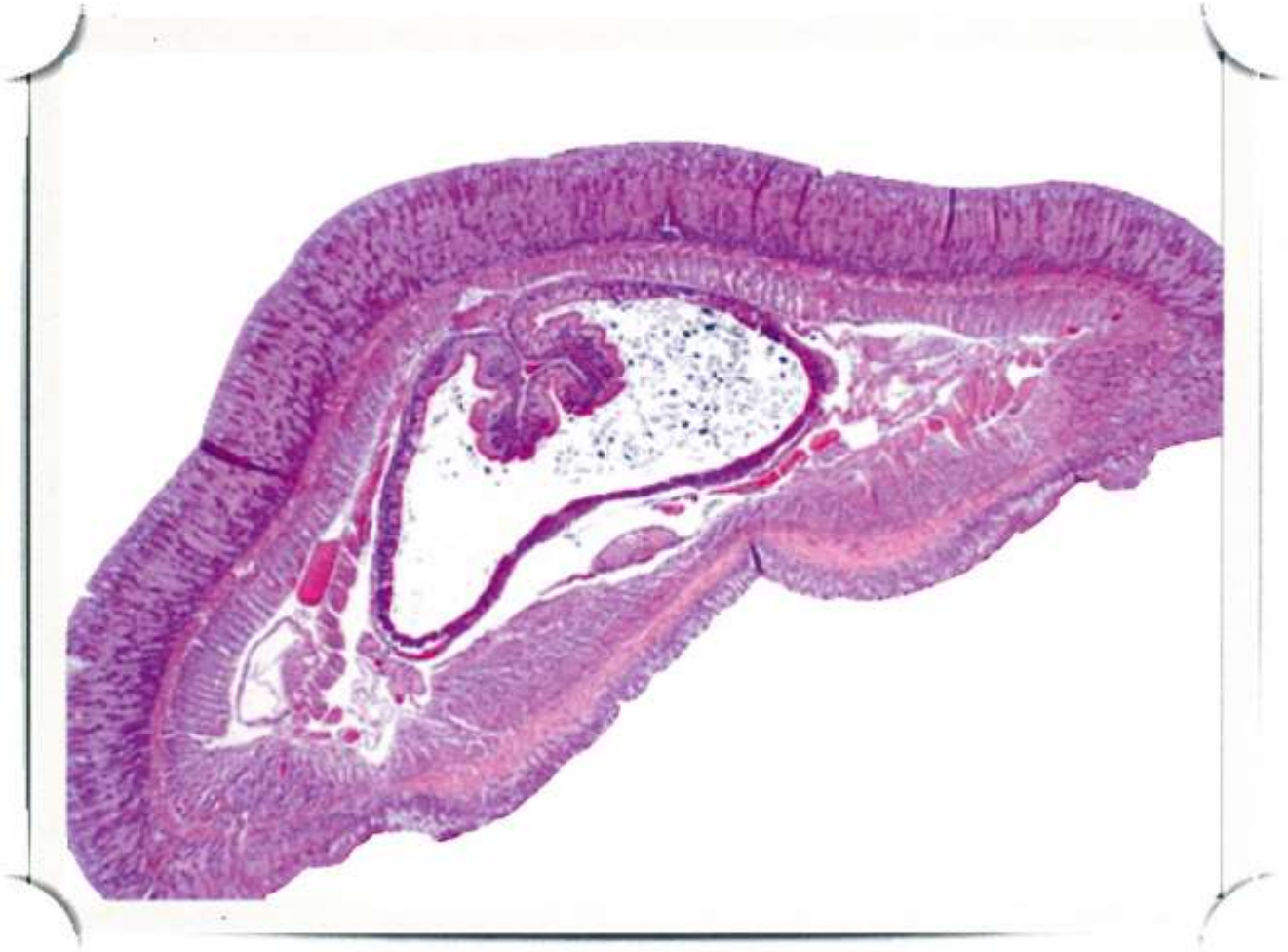
The effect of nerve regeneration on nerve function and structure

James Gabor



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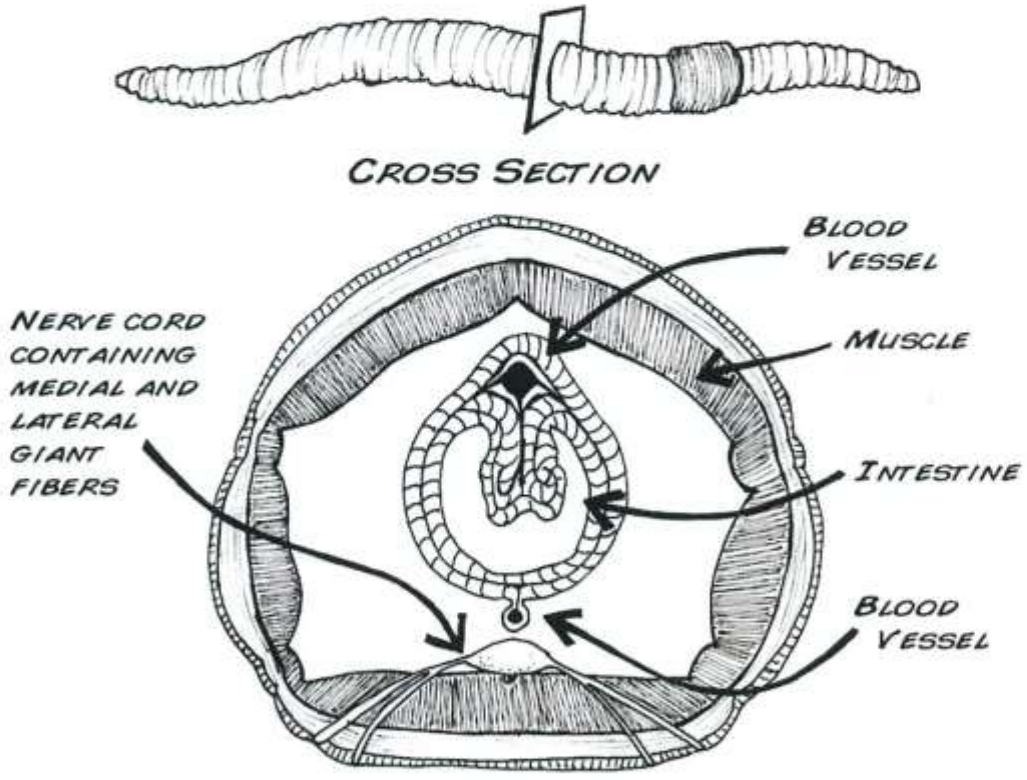
Background Information

Earthworms are invertebrate animals, with a relatively simple nervous system compared to vertebrates. Rather than having a "brain," the earthworms nervous system is controlled by its cerebral ganglion[1] (a large cluster of nerves in the anterior of the worm). The main nerve cord (the ventral nerve cord, VNC) is attached to the ganglion and runs length wise down the body.

From the VNC, separate nerves, which are called segmental ganglions, extend into different parts of the worm and are responsible for movement (Figure 1). These segmental ganglia are sensory in function, sensitive to touch and temperature.

Figure 1 Schematic anatomy of the worm



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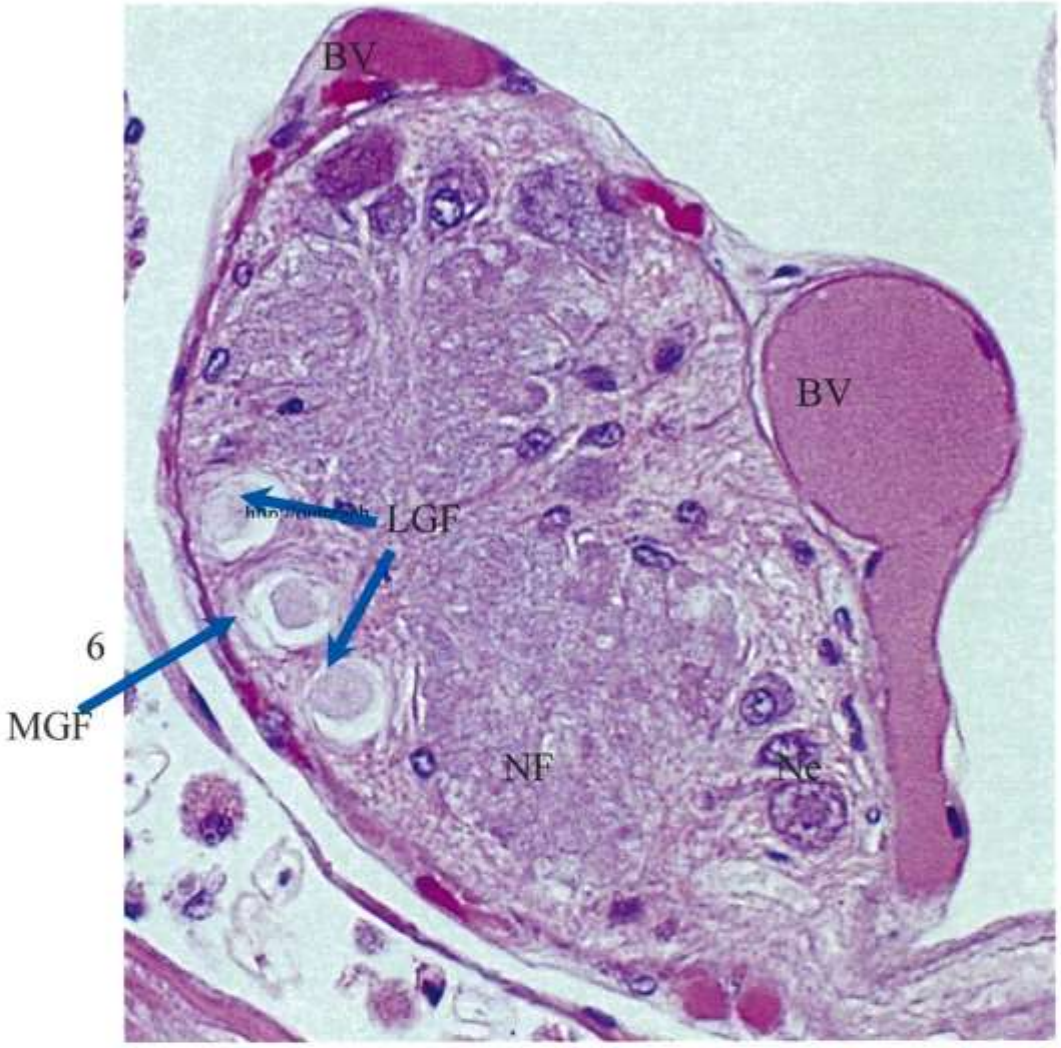
Following sensory stimulation (light, touch or heat), segmental ganglia conduct the signal to the ventral nerve cord and the worm responds by contracting its muscles.

The VNC contains a single median giant fibre (called the medial giant axon - MGA) and two lateral giant fibres (lateral giant axons - LGA) surrounded by clusters of neurons (ganglia) including sensory, motor and interneurons. The median fibre is a sensory nerve detecting touch stimuli from the anterior segments (the front of the worm) and the two lateral fibres detect touch stimuli to the posterior segments (the back of the worm). The lateral fibres operate as a single functional unit by forming numerous neural connections. The signals that are sent to the cerebral ganglions contain all information concerning the environment around the worm.[2]

In Figure 2, which is a histological image of the VNC of a worm, the median giant fibre and the two lateral giant fibres are clearly apparent. The median giant fibre conducts impulses from the anterior segments while the lateral giant fibres conduct impulses as a single unit from the posterior segments.

Figure 2: Cross section of worm ganglion



MGF - Median Giant Fibre LGF - Lateral Giant Fibre

BV - Blood Vessel NF - Nerve Fibre

Ne - Neuron

Worms are able to regenerate if they lose a part of their body. However, they can only regenerate if the cut is at least 10 segments below the saddle or clittelum[3]. Any cut above that results in death of the worm. Regeneration differs from growth as it involves the replacement of a severed part rather than an increase in size. It should be expected therefore that regeneration will involve some sort of altered function and or structure.

Nerve regeneration is an important and emerging field of human neuroscience. Traumatic injury, infections and other diseases can all cause damage to nerves, and in most cases, successful regeneration does not occur. This often results in the loss of a bodily function and a loss of

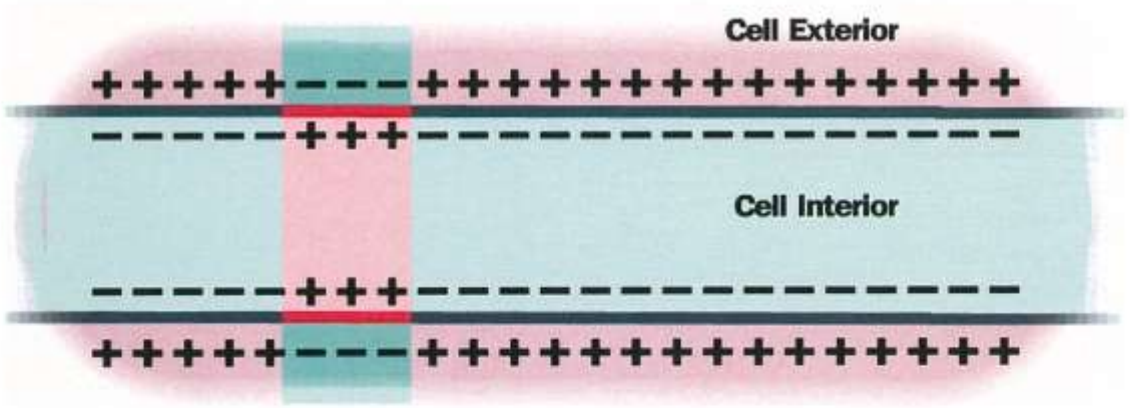
quality of life. This may be due to the complexity of the human body making a full regrowth difficult. The ability to stimulate nerve regeneration requires a fundamental understanding of the processes involved and therefore, the use of invertebrates capable of nerve regeneration are a valuable experimental model.

Any organism with a nervous system must be able to communicate. Messages are sent from the brain (in the case of an earthworm, its cerebral ganglion) to particular parts of the body to perform certain actions such as movement. These messages are transmitted throughout the worm by nerves (clusters of neurons). Each neuron has a long extension, or axon which transmits the message via an electric signal. The purpose of the axon is to conduct electrical messages called action potentials and pass this signal to other contact neurons[4].

Axons are surrounded by glial cells which insulate axons (in vertebrates, some larger nerves are surrounded by modified glial cells called Schwann cells which produce a myelin sheath). This insulation acts to increase the speed of action potentials. An action potential is simply an electrical charge traveling down the axon of a neuron. An action potential, however, can only occur when there there is a relative difference in charges on either sides of the cell membrane. The outside of a neuron's axon is positive relative to the inside of the cell, which is relatively negative. An action potential is a rapid reversal of the charge which flows down the entire length of an axon (Figure 3)[5].

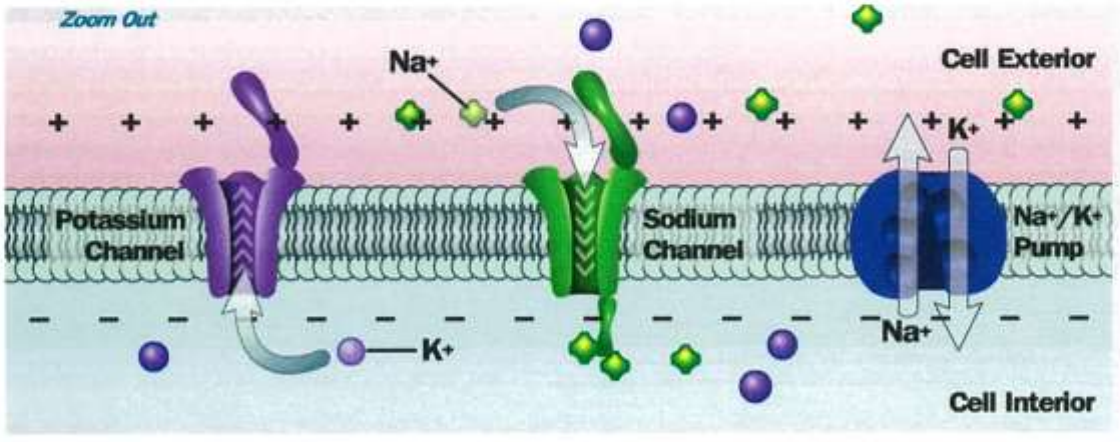
The difference in electrical charges across the cell membrane is called polarity and is created by ions inside and outside the cell. The main ions used to generate polarity are sodium ions (Na^+) and potassium ions (K^+).

Figure 3: Schematic diagram of an action potential (AP) in an axon.



Axons have many channels that allow only Na^+ or K^+ to pass through. When the neuron is at rest, or not transmitting any electrical messages, Na^+ and K^+ ions move down their channel to the opposite side of the membrane. Meanwhile, a specialised pump (Figure 4) located in the axon forces sodium and potassium back to the sides they came from. For every three sodium ions pumped outside the neuron, two potassium ions are pumped in. This results in an increase in positive charges on the outside of the axon compared to the inside.

Figure 4: An axonal membrane with Na, K and Na/K pumps



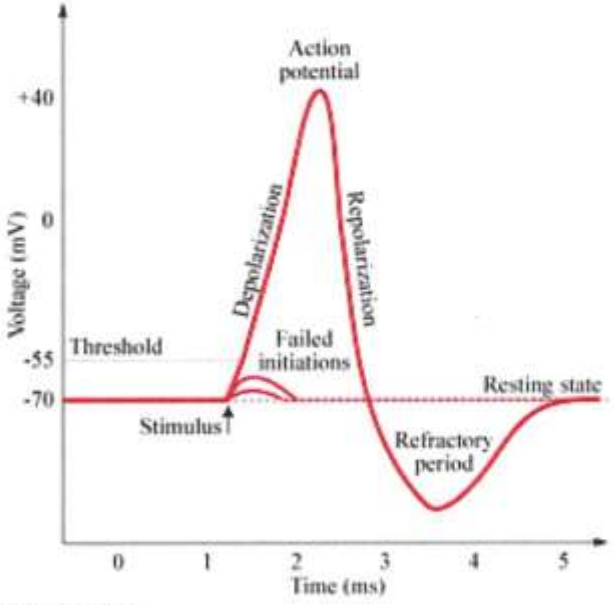
http://outreach.mcb.harvard.edu/animations/actionpotential_short.swf

The result is that the outside of the neuron is positively charged while the inside is negatively charged. It is estimated that all organisms use 30% of their energy to maintain this polarity across all cells in the body.

An action potential is an instantaneous change in this charge (Figure 5). For an action potential to be sent, the inside of the cell must become positive (depolarization). For this to occur, the sodium channels open allowing sodium ions to rush in making the interior positive. The next step is repolarization which is returning the cell to its original state where the inside is relatively negative.The sodium channels close, and the potassium channels open so K flows into the cell. The return to resting potential occurs with closure of the K channel and the Na/K pump restoring the positive charge to the outside of the cell and negative on the inside by moving 2 K^+ ions in, but 3 Na^+ out.

This entire process occurs in a fraction of a second and occurs down the entire length of the axon. Often, the electrical message is sent to other neurons which send the action potential down their axons until the message reaches its target. If the signal is not strong enough, a failed initiation will occur where the potassium pump will attempt to close but will not. Action potentials can be triggered by any sort of interaction with the nerve.

Figure 5 Graphic representation of an action potential.



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In this experiment, I will be testing the hypothesis: does the nerve function, measured by the speed of the electric current (action potential) or nerve structure (histology) alters during the process of nerve regeneration in an earthworm.

Experiment

Does nerve regeneration effect function (action potentials) and structure (histology)?

Hypothesis

My hypothesis is that the action potential of the regenerated nerve will be slower (the action potential length will be increased) than the nerve of the intact earthworm and that this will be reflected by structural differences in the regenerating nerves.

Aim

To determine how nerve regeneration of an earthworm affects nerve function (action potentials) and structure (histology).

Variables

1. The independent variable is the nerve.
2. The dependent variables are the action potential length and the histological appearance.
3. Variables kept constant are size of the worm, maturity (presence of a clitellum), physical environment (container, food, lighting, moisture), length of time (treated at same time, dissected at same time, killed at same time).

Equipment

- Worms x20
- 2 meters plywood
- Roll of wire mesh
- Plastic mesh
- Large plastic container x2
- Saw
- Staple gun
- Spikerbox
- Headphone cable
- Styrofoam
- Ruler
- Paper towels
- Scalpel
- Computer (with the application audacity installed)
- Ethanol (10%)
- Latex gloves
- Bouins fixative
- 10% Neutral buffered formalin
- Large plastic bottle x2
- Histological processing equipment
- Water
- Faraday cage (to prevent outside noise from influencing action potentials
- Slide cassettes x40
- Tweezers

Risk Assessment

- While constructing the faraday cage, I used a staple gun and a saw. These tools both have a level of risk. These tools have a medium level of risk as they can cause bodily damage but not to a severe level. There is medium level chance that an accident could occur with these tools. Knowing the correct method of using the tools and adult supervision is the best method to minimise this risk.
- Use of ethanol. Ethanol is a highly flammable and toxic substance which I used to anesthetize the worms. If it touches your skin or gets in your eyes, stinging and burning can occur. If inhaled, it can cause irritation to the eyes or throat and if ingested at low or medium concentrations, can cause blurred vision and blackouts. If ingested at a high concentration, can cause convulsions, comas and breathing complications. This is a high risk substance and the best way of reducing an accident is to use it at a low concentration (10%), keep it in a safe place, with a secure lid, have adult supervision and wash hands directly after use.
- Use of bouins and 10% neutral buffered formalin fixatives. Both fixatives are highly dangerous, highly toxic chemical substance which can cause serious bodily harm. It also has a long term effect as it is also carcinogenic. To reduce the chances of being harmed, double gloves were worn as well as having parental guidance and assistance. Hands were also immediately washed following the use of the fixative. A way

to further reduce the risk would be to wear a face mask or use the fixative in a fume hood.

Method

1. Preparing the worms for testing and dissection

I. Gather 20 worms of approximately the same size from the worm farm. Make sure that the each have a clittelum as this will be a benchmark of maturity and where to dissect to ensure proper regrowth.

Figure 6: Selection of worms from compost bin

II. Measure worm size. Note: as worms contract muscles, worm measurements are not completely accurate, so allow the worm to relax before measuring.

III. Place each worm into container layered with damp paper towels to ensure that the worms do not dry out. Leave the worms for 48 hours to clear intestinal contents prior to histological examination.

1. Construction of faraday cage

I. Acquire 4 meter 1.5 X 1.5 plywood, wire meshing, saw and a staple gun

II. Measure out an 8 X 16 inch rectangle on the mesh

Figure 7: tools to make a Faraday cage

III. Cut out the rectangle

IV. Cut out five 8 inch pieces of plywood

V. Unroll the mesh so that it lays flat on table to make it easier for stapling

VI. Take the first wood strip and staple it to the underside of the end of the mesh rectangle

VII. Staple the next wood strip 5.5 inches down from the first woodstrip

VIII. Staple the third wood strip 2.5 inches distant from the previous wood strip

IX. Staple the fourth wood strip 5.5 inches distant from the third wood strip

X. Staple the final wood strip at the end of the mesh

XI. Shape the metal mesh into a cage, using the stapled wood strips as braces so that the mesh keeps its position

Figure 8: Finished Faraday cage

2. Setting up equipment for first stimulation

I. Download audacity, a free program to record the action potentials of the worm

II. Plug in the audio cable to the computer and the spikerbox

III. Using blue tack, stick a 4 cm piece of styrofoam to the top of the spikerbox

IV. Plug in the recording electrode into the spikerbox

V. Create a 10% solution of ethanol to anesthetize the worm during experiment

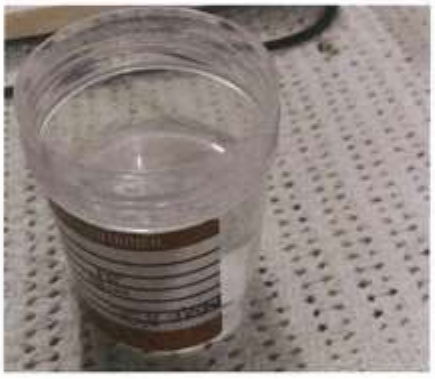
VI. Place the spikerbox into the faraday to block out any outside noise

VII. Place a second container layered with damp paper towels nearby

3. Stimulation of worms before regeneration

I. Place worm into 10% ethanol solution for 1 minute

Figure 9: 10% ethanol for anesthetizing worms



II. Remove worm and place onto styrofoam on spikerbox

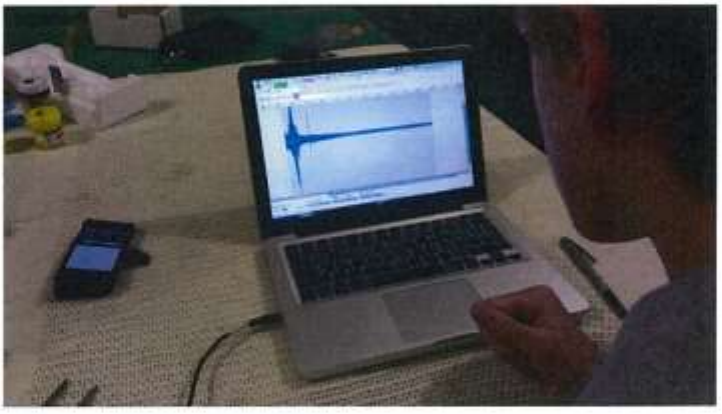
III. Stick the recording electrodes into the worm

Figure 10: Measuring worm on spiker box

IV. Measure the worm and record length

V. Begin the recording using audacity and prod the posterior of the worm five times with a toothpick

Figure 11: recording action potentials



VI. Remove the worm from the spikerbox and place into second container.

VII. Repeat for next two worms

VIII. When it is the fourth worm, increase the amount of time in ethanol solution to 1 minute 30 seconds.

IX. When it is the sixth worm, leave in ethanol for 2 minutes

X. When it is the tenth worm, pour out ethanol solution and create another 10% mixture

XI. Leave the worm in for 1 minute and repeat previous steps

XII. Once all 20 worms have been recorded, individually place each worm on table to prepare for bisection

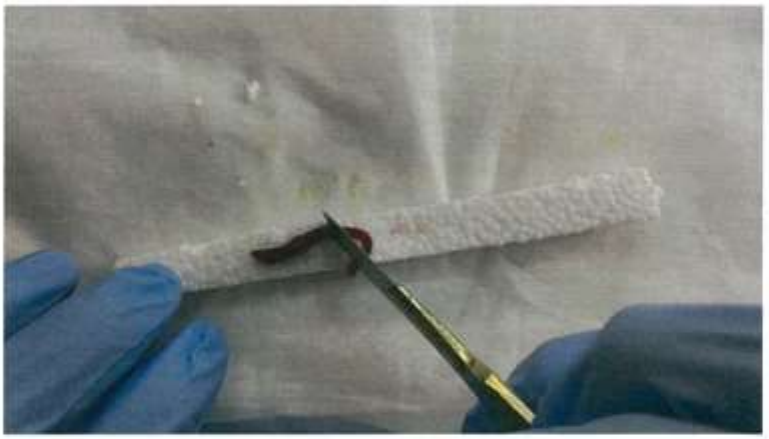
4. Dissecting the worms and using the bouins fixative

I. Using a scalpel and while wearing gloves, carefully cut the worm ten segments below the saddle

II. Place the anterior half of the worm into a container with moist fresh compost

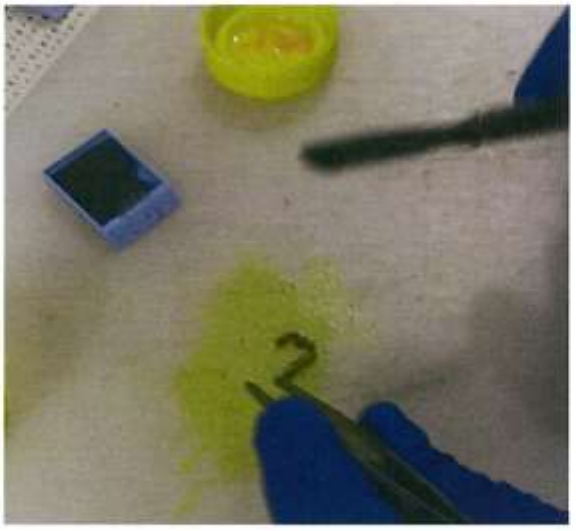
III. Place the posterior of the worm into a bouins fixative for 30 minutes to fix the worm

Figure 12: Bisection of the worm below the clittelum



IV. Repeat for all 20 worms

Figure 13: Trimming worms into segments to place in histological cassette



V. Using tweezers, remove the posterior end of the worm from the bouins fixative

VI. Using a scalpel, carefully cut the rear of the worm into multiple pieces for the histological photo

VII. Place the segment of the worm into a slide cassette with its number clearly labelled

VIII. Repeat for all worm posteriors

IX. Once all worm segments are inside labelled cassettes, place them into the 10% neutral buffered formalin fixative and leave for an hour

Figure 14: Fixing worms in 10% neutral buffered formalin

X. Remove the cassettes from the fixative

XI. Cassettes are now ready for histological processing. This step was completed in The State Diagnostic Laboratory by a Histology Technician. The simplified process involves

a. Removal of formalin and replacement with alcohol

b. Gradual replacement of tissue fluids with chemical fixatives and paraffin wax

c. Embedding the tissue in wax (to hold it rigid)

d. Cutting 5 micrometer sections with a microtome and placing on a glass slide.

e. Staining with haematoxylin (for a blue coloration of tissue) and eosin (pink).

f. Photos were taken of ventral nerve cord from 5 worm cross sections.

5. Regrowth period

I. Go back to compost bin

II. Sieve through the compost and remove any worms hidden inside the waste

III. Place the compost into the container with all the anterior (front) parts of the worm

IV. Acquire plastic mesh and cut out to fit on top of container

Figure 15: Preparation of container for worms

V. Put damp paper towels over the compost

VI. Leave container in a dark room with a relatively constant temperature.

VII. Every second day, remove paper towels and replace with new damp ones

VIII. Every week, remove compost and refresh with new compost, again sieve through the waste to remove any worms

IX. Worms were maintained for one month before restimulation.

6. Stimulation of worms after regeneration

I. Repeat step 3 and 4

II. Once all worms action potentials have been recorded, it is time to place the worms into another bouins fixative for their final histological processing.

III. Place each worm into another bouins fixative for 10 minutes

IV. After ten minutes, remove a worm and with a scalpel, cut into ten segments and place into a labelled slide cassette

V. Repeat for all worms

VI. Once all worm segments are inside labelled cassettes, place them into the bouins fixative and leave for an hour

VII. Remove the cassettes from the fixative

VIII. Cassettes are now ready for histological processing as detailed above.

8. Evaluation of results

I. For each stimulated worm, five action potentials were selected and files saved for evaluation.

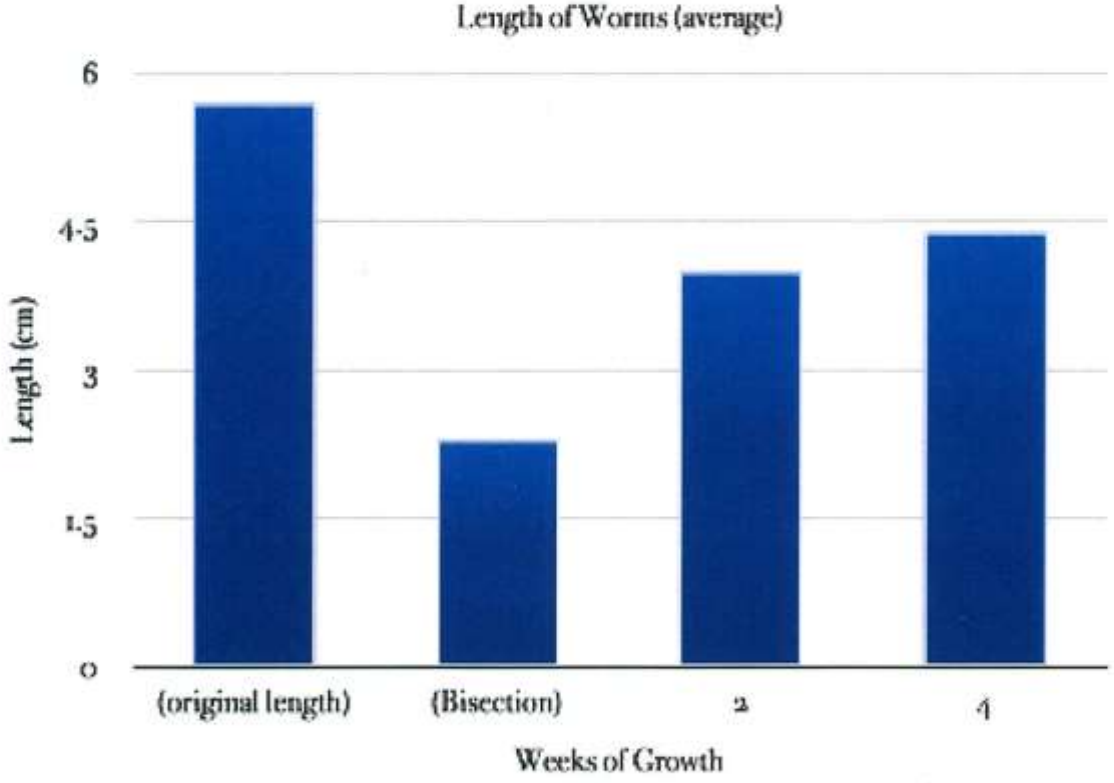
II. For each histological slide, the ventral nerve cord from 5 worms were selected and photographed.

Results

Physical measurements

Twenty worms were collected with an average length of 5.7 cm (Graph 1). All worms were highly responsive to light touch with muscular contraction. All had a clearly visible clittelum. Following bisection, all worms had an average length of 2.3 cm and moved when touched. Two weeks later the average length of the bisected worms was 4 cm, however three worms could not be found, and were presumed perished. Four weeks later the worms were an average of 4.4 cm and were responsive to touch with muscular contraction. The regenerated segment was lighter in colour and the tip of the tail was visibly thinner. This represented the newest tissue.

Graph 1: Average length of worms prior to bisection and during regeneration.



Action potentials

The action potential is divided into 5 clearly identifiable phases. These were all evident in the worms examined with five action potentials being examined per worm.

Each action potential had a rising phase (depolarization), the peak phase (depolarization stops), the falling phase (repolarization, becoming more negative), the undershoot phase (temporary membrane potential more

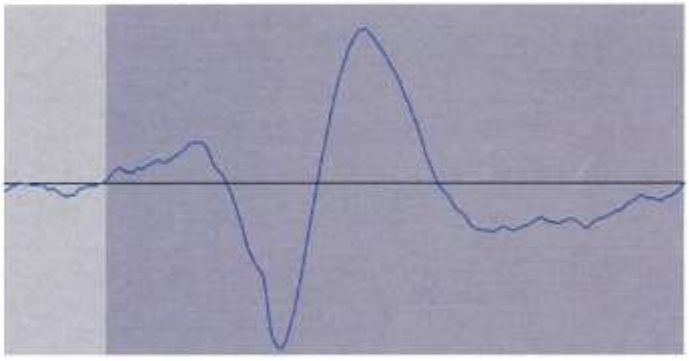
negative then at rest) and finally the refractory phase which involves the leveling out of the action potential. This usually causes another jump above the resting point and another undershoot phase.

The light grey area before the action potential begins is the resting state (Figures 16, 17). The reason why it is not completely flat is that there are a number of failed initiations which is where the electrical signal is not strong enough to begin an action potential.

Before regeneration:

The action potentials from 20 worms were recorded before bisection and regeneration and were of normal structure (Figure 16). The length of these action potentials before regeneration varied between 4×10^{-3} seconds and 7.5×10^{-3} seconds, with an average action potential length of 5.1×10^{-3} seconds (Table 2). The mean and standard deviation of the worms before bisection was $5.1 \pm 0.8 \times 10^{-3}$ seconds. The action potentials were easily generated by light touch to the tail with a toothpick.

Figure 16: representative action potential in worm before bisection



After regeneration:

The length of the action potentials of the 17 worm after bisection and regeneration varied between 4×10^{-3} seconds and 8×10^{-3} seconds. The average action potential length was 5.2×10^{-3} seconds. The mean and standard deviation of the worms after bisection was $5.2 \pm 1.1 \times 10^{-3}$ seconds. Greater pressure was required to generate the action potentials with more prodding required when compared to worms before bisection.

Figure 17: Representative AP after bisection (regeneration)

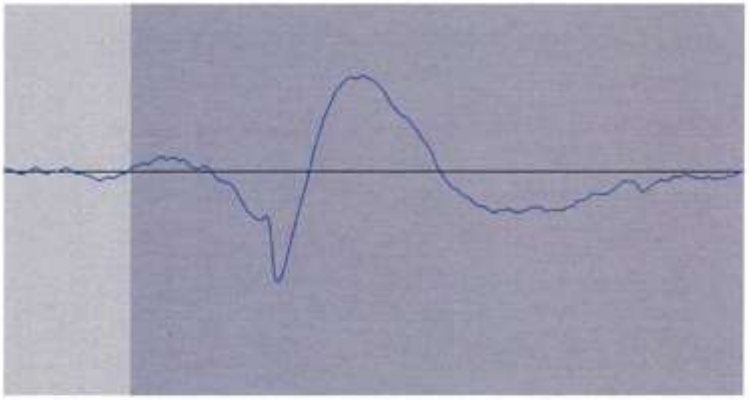


Figure 17 is an example of an action potential from a worm after regeneration. The action potential has clearly defined phases and is of comparable duration to that seen in Figure 16. However one difference is that the undershoot phase drops at a faster rate and that the overall intensity of the action potential isn't as strong.

No significant difference was found in the action potentials when comparing the worms before and after bisection (Graph 2).

Graph 2: Scatter plot of average action potential length before and after bisection.

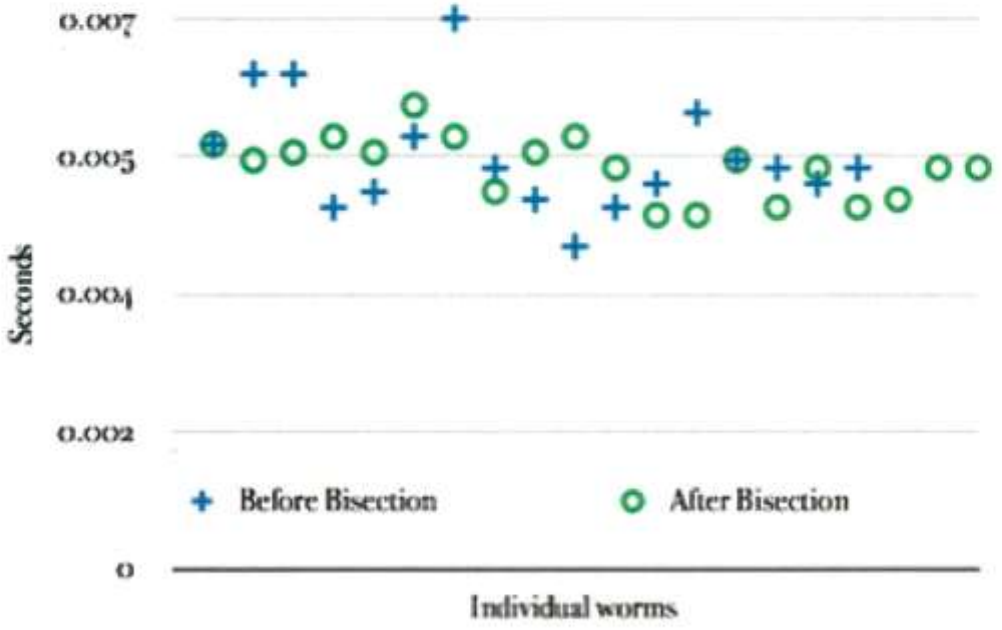


Table 2 - comparison of average AP time before and after bisection

Individual worm	Average AP time before (seconds)**	Average AP time after (seconds)**
1	0.0054	0.0054
2	0.0052	0.0063
3	0.0053	0.0063
4	0.0055	0.0046
5	0.0053	0.0048
6	0.0059	0.0055
7	0.0055	0.0070
8	0.0048	0.0051
9	0.0053	0.0047
10	0.0055	0.0041
11	0.0051	0.0046
12	0.0045	0.0049
13	0.0045	0.0058
14	0.0052	0.0052
15	0.0046	0.0051
16	0.0051	0.0049
17	0.0046	0.0051
18	0.0047	-
19	0.0051	-
20	0.0051	-

**raw data from 5 AP per worm are presented within the appendix

Histological results

Each cross section was examined and the ventral nerve cord identified. In some instances nerve fibres extending to/from the muscle layer could also clearly be seen.

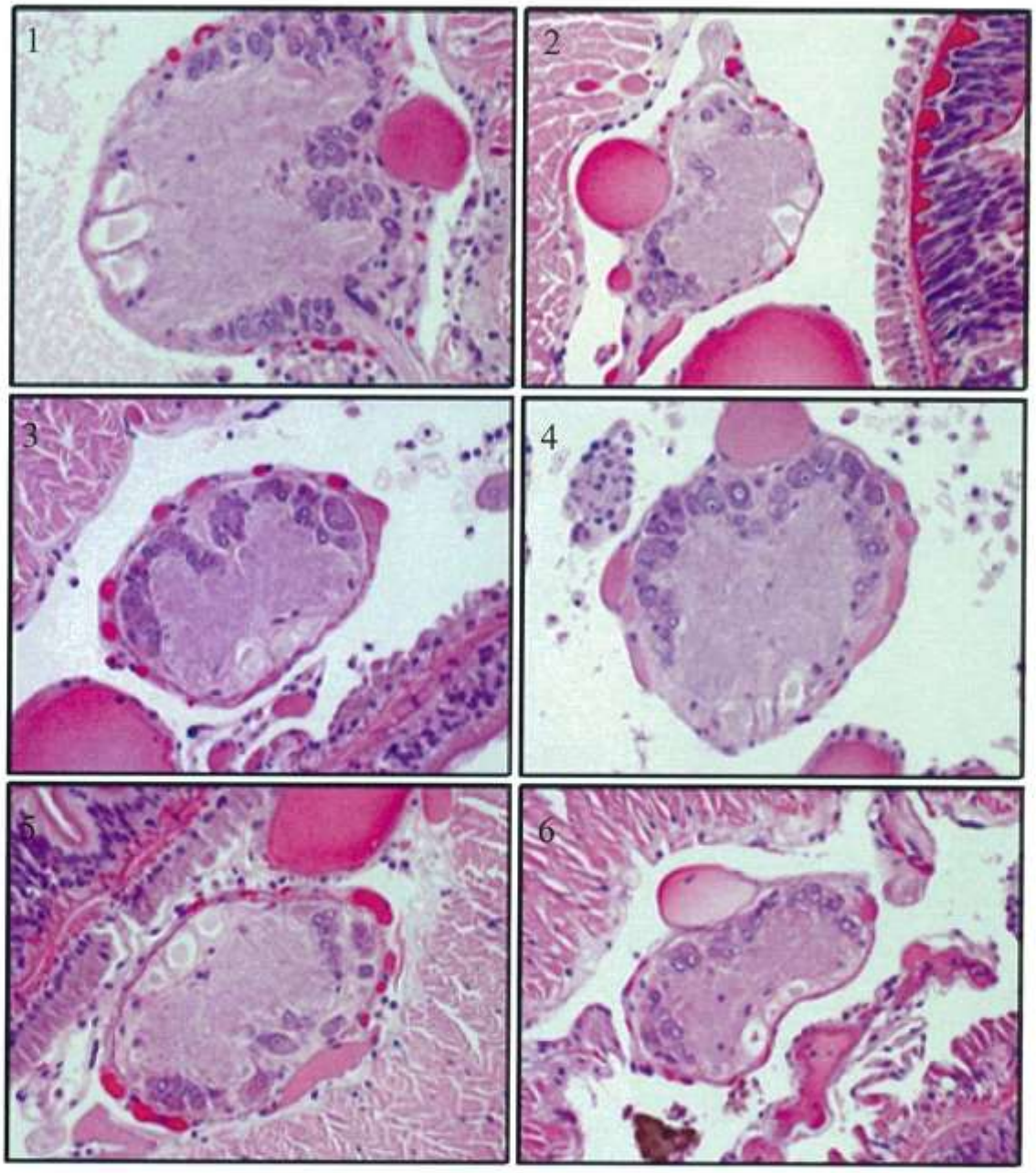
Before regeneration:

In the 20 worms examined before regeneration, 5 representative photos are taken of 5 ventral nerve cords. In each cross section (Figure 18), the medial giant axon and two lateral giant axons are faintly eosinophilic (pink in colour), finely granular (slightly dotty appearance) and all three giant axons are similar in diameter and size. Each of the three axons are surrounded by a sheath consisting of clear space. The center of the ventral nerve cord consists of dense tangles of fine nerve fibres which are slightly more eosinophilic than the giant axons.

Numerous nuclei of the neurons are located at the top (dorsal) surface of the ventral nerve cord forming two well defined arches. The neurons have a distinctive basophilic (blue color) cytoplasm with a central nucleus and a prominent, deeply basophilic nucleolus. The entire ventral nerve cord is surrounded by support cells and many dilated blood vessels. In some images, nerve fibres can be seen extending to/from the surrounding muscle layer (Figure 18 - image 1). In these cases the fibres are deeply eosinophilic and aligned in parallel.

These findings were consistent in the all histological photos of the worms before regeneration. Figure 18 shows 6 representative cross sections from 6 different worms.

Figure 18 : Histological appearance of ventral nerve cord in 6 representative worms before regeneration



After regeneration:

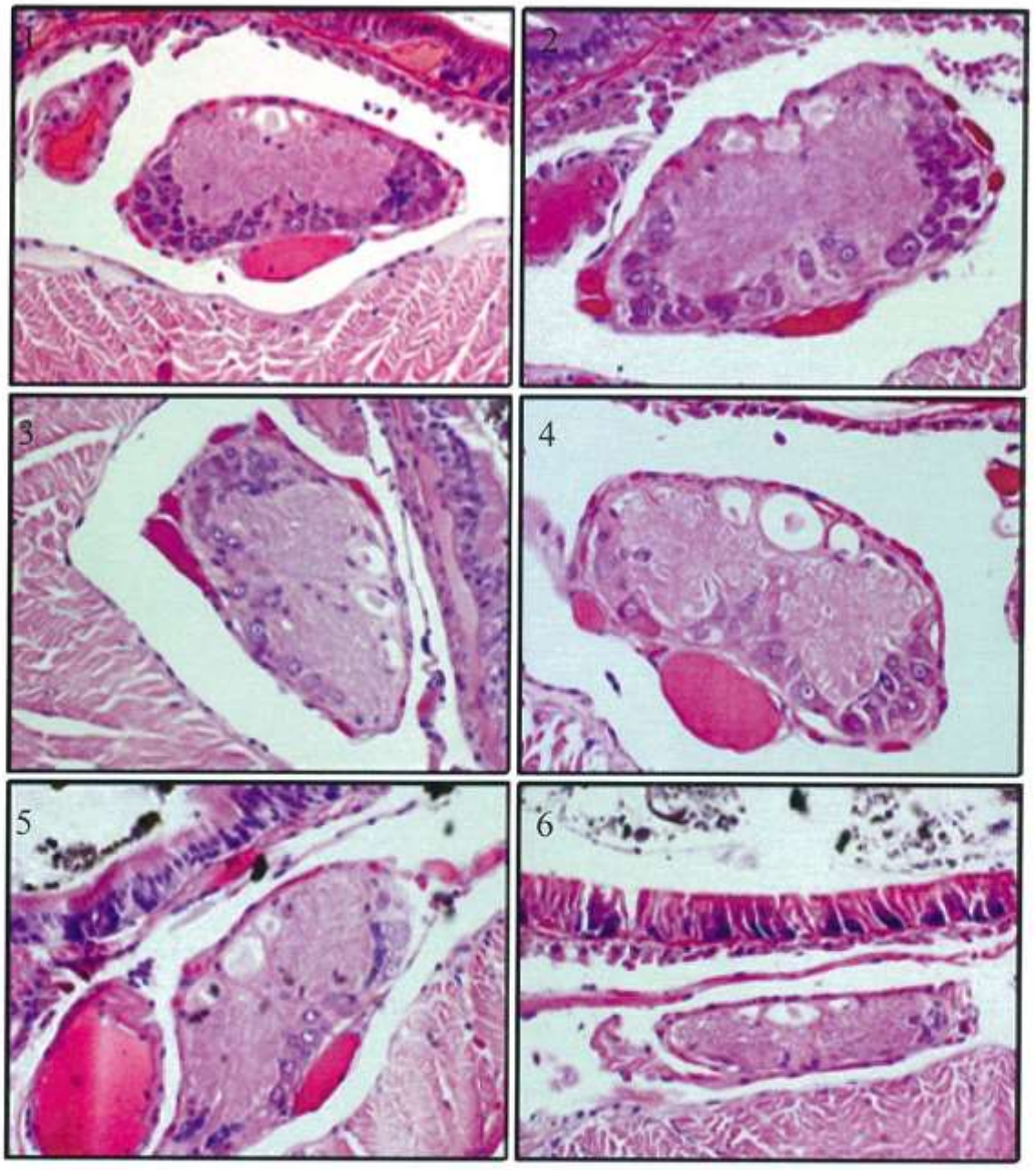
In the 17 worms examined after regeneration, the cross sections closest to the clittelum are very similar histologically to the cross sections observed prior to bisection Figure 19, photos 1, 2). These sections are the first to regenerate and therefore the oldest. Most significantly, the medial and lateral giant axons are of similar size, colour and granularity. The clusters of neurons are of similar to density to those before regeneration and formed distinctive arches.

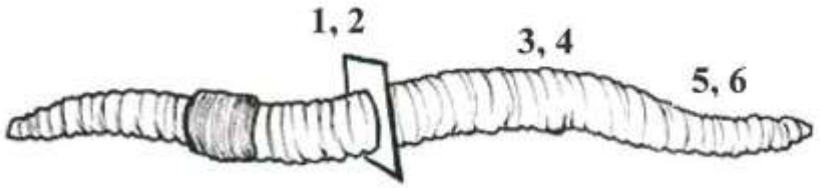
In contrast, cross sections from closest to the tip of the tail had a distinctly altered histological appearance (Figure 19 photos 5, 6). In each cross section, the medial giant axon and the two lateral giant axons are smaller, irregular in profile, more eosinophilic in colour and more densely granular than in the before bisection group. The diameter of the lateral giant axons is considerably smaller, more granular and often lacking structure. In these sections, often only fibres can be seen instead of a full axon. The nerve fibres are more eosinophilic and the neurons often did not form a complete set of two arches.

Neurons are present in reduced numbers or barely present in the after group. While in the photos from areas which have had multiple weeks to grow have a number of nuclei, the areas still in development only have a few and do not clearly form the two arches which is present in the before group (Figure 19 photos 1, 2). Figure 19 photos 3, 4 show cross sections with intermediate changes.

In all sections examined, nerve fibres can be seen extending to the surrounding muscle layer.

Figure 19: Histological appearance of ventral nerve cord in 6 representative worms after regeneration (cross sectional cuts noted in schematic below)





Discussion

This experiment was designed to look at the effect of nerve regeneration on the function and the structure of the regenerated nerve. While it was predicted that the function would be impeded, with supporting evidence from the structure of the nerve, this was only partly correct. The results have shown that there was no change in the length of the action potential. This demonstrates that the earth worm is able to regenerate their nerves without lowering the speed of the electrical conduction. However, from studying the histological photos, it was also clear that the regeneration process involved structural changes to the VNC.

From studying the histological photos of the worms before and after the bisection, it is obvious that the nerves had not regenerated to their original state and were now incomplete and immature. This is clearly a time dependent process as segments regenerated first had a more mature appearance. However those segments which had the least amount of regeneration time (those near the tail) were immature in appearance with decreased cellular and fibre density. It is also obvious that the lateral giant axons were the slowest to regenerate as they were noticeably smaller than the medial giant axon. This could be a reflection of its more complicated structure and the likely cause of the reduced responsiveness when the posterior was stimulated. As an aside observation, light stimulation of the anterior end did generate an action potential.

Surprisingly though, despite the immature histological appearance and the fact that a greater stimulus was required to generate an action potential compared to the untouched earthworm, the action potentials were in fact as fast as the untouched worm. That is, the threshold was altered but not the process of propagating the action potential. This ensures that the worm's interaction with its environment is not affected and that it is able to respond to different stimuli. During an action potential, the sodium and potassium can either be opened or shut. It is an instantaneous process which does not appear to be affected by the regeneration of nerves.

The histological appearance of the regenerating nerve of the earthworm differed from what is seen in vertebrate nerve regeneration (such as humans). In the worm the structure was normal, but lacked maturity. In contrasted, nerve regeneration/repair in vertebrates shows disorganisation and sprouting of new fibres. A recent article in Neuron[6] has identified the signals used by worms during regeneration and suggests that these pathways are prevented or inhibited in humans.

Complete regeneration appears to be an effective process in invertebrates. In an early experiment[7], it was shown that if an incision was made in an earthworm and the nerve was only cut, not the entire worm as in this

experiment, the speed of the action potential would only be a fraction of its original speed. With my experiment, the worm was cut in half and the nerves were allowed to regenerate with no change length of the action potential. This means that full regeneration of a worm is more effective and likely involves a different process than the repair process that follows the cutting of a nerve.

From this, we are able to deduce that the worm, while simple in appearance, is an incredibly complex creature capable of entirely regrowing a nerve with minimal loss of function. The amazing part of this is that the area which was dissected didn't grow back as repair tissue, scar tissue or cartilage but as a fully functional nerve.

My experiment used a large cohort of worms, with good control of variables. This has produced a strong set of results which could be expanded on in future experiments.

Future directions

While the experiment did explore and analyse the functions of the nerve in an earthworm and how it functions after regeneration, it could have been expanded further and there could have been small improvements.

I chose to only stimulate the posterior of the worm and therefore only looked at the function of the lateral giant axons. To also stimulate the anterior of the worm and measure the action potential in the medial giant axon would add additional valuable information on regeneration. Additional investigation into the different processes of the nerve regeneration would also have been interesting. The use of special stains for myelin, longitudinal cuts of the nerve and sampling at more time points would have provided a more in depth analysis of the earthworm if it was implemented.

Similarly, measurement of the action potentials at earlier time points may have revealed a time when action potentials could not have been generated. I would suggest weekly measurements; however the impact on the worm would have to be taken into account. Anaesthetic, electrode placement and stimulation are all interventions which could potentially affect the health of the earthworm. This would have given an idea of what the action potentials were like during early regeneration.

It has recently been shown that the ability for nerves to regenerate in the worm is lost as the worm ages. This is due to the decline in an essential regeneration promoting factor[8]. Further experimentation could explore at what age the ability of nerve regeneration is lost in earthworms.

All the results gathered concerning the independent variable (the nerve) were measured by way of the dependent variables (histological photos and

action potential measuring). All other variables were controlled to minimise the effect on the dependent variables. A large sample size was used to ensure statistics could be used and valid.

Conclusion

My hypothesis was that nerve regeneration following bisection would result in an altered action potential length in the regenerated nerve which would be a reflection of altered nerve structure.

The results demonstrate that, although the nerves were less responsive to stimulation, the action potential length was not altered in the regenerating nerve. Additionally, there was a marked difference in the structure of the nerve, most noticeable within the lateral giant axons.

Appendix

Table 2a - Individual and mean action potential (AP) times before bisection

Individual Worm	Individual action potential length BEFORE BISECTION (sec) (five AP per worm)				
1	0.0050	0.0055	0.0055	0.0055	0.0055
2	0.0060	0.0045	0.0050	0.0053	0.0050
3	0.0053	0.0047	0.0060	0.0050	0.0055
4	0.0059	0.0055	0.0055	0.0055	0.0050
5	0.0050	0.0055	0.0050	0.0055	0.0055
6	0.0065	0.0043	0.0055	0.0075	0.0055
7	0.0060	0.0050	0.0065	0.0045	0.0055
8	0.005	0.0050	0.0040	0.0050	0.0050
9	0.0074	0.0047	0.0048	0.0045	0.0050
10	0.0053	0.0039	0.0074	0.0053	0.0056
11	0.0040	0.0050	0.0075	0.0044	0.0045
12	0.0043	0.0040	0.0060	0.0065	0.0052
13	0.0042	0.0050	0.0044	0.0044	0.0046
14	0.0043	0.0056	0.0051	0.0043	0.0065
15	0.0055	0.0043	0.0044	0.0040	0.0050
16	0.0050	0.0042	0.0044	0.0075	0.0046
17	0.0053	0.0048	0.0044	0.0043	0.0040
18	0.0041	0.0046	0.0045	0.0050	0.0055
19	0.0060	0.0045	0.0050	0.0052	0.0050
20	0.0047	0.0054	0.0048	0.0050	0.0055

Table 2b - Individual and mean action potential (AP) times after bisection

Individual worm	Individual action potential length AFTER BISECTION (sec) (five AP per worm)				
1	0.0060	0.0050	0.0055	0.0055	0.0048
2	0.0040	0.0080	0.0057	0.0070	0.0068
3	0.0046	0.0070	0.0066	0.0065	0.0069
4	0.0045	0.0045	0.0042	0.0042	0.0040
5	0.0052	0.0046	0.0044	0.0047	0.0050
6	0.0055	0.0050	0.0054	0.0058	0.0060
7	0.0076	0.0083	0.0060	0.0062	0.0068
8	0.0051	0.0050	0.0049	0.0062	0.0045
9	0.0044	0.0060	0.0036	0.0040	0.0056
10	0.0035	0.0052	0.0040	0.0040	0.0040
11	0.0056	0.0043	0.0045	0.0042	0.0045
12	0.0040	0.0040	0.0075	0.0046	0.0045
13	0.0067	0.0072	0.0057	0.0051	0.0045
14	0.0048	0.0049	0.0051	0.0053	0.0058
15	0.0045	0.0045	0.0045	0.0045	0.0075
16	0.0050	0.0052	0.0045	0.0048	0.0052
17	0.0049	0.0054	0.0054	0.0045	0.0052
*	-	-	-	-	-
*	-	-	-	-	-
*	-	-	-	-	-

(*3 worms did not survive to final testing)

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