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Muscle Contraction in the Pharyngeal Portion of *Caenorhabditis elegans* is Effectively Anesthetized by (-)-Tetramisole Hydrochloride

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II. Introduction

Caenorhabditis elegans are transparent multicellular worms (nematodes) with simple, distinct organ systems (Wharton, 1986) containing nine hundred and fifty nine cells, excluding eggs and sperm, or about 2,000 including the gametes, identical in all *C. elegans* (Brown, 2003). They are ideal creatures to observe due to several features that they exhibit. Aside from being transparent, which eliminates the need for invasive techniques; *C. elegans* have a short generation of time of only 3.5 days (Wharton, 1986). In addition, the nematode can reproduce hermaphroditically, allowing the production of up to 100,000 descendants in ten days (Brown, 2003). The worm is also easy to culture and is free living (Wharton, 1986).

The alimentary canal of the *C. elegans*, beginning at the mouth on the anterior end and ceasing at the anus at the posterior end is associated with only esophageal and anal dilator muscles; the rest is controlled entirely by turgid pressure (Wharton, 1986). The esophageal pump is ideal for nematodes, which mostly feed on liquids (Wharton, 1986), filtering out the necessary nutrients and expelling the rest of the water (Brown, 2003). The esophagus is involved not only in pumping, or swallowing food down the alimentary canal, but it also assists in filtering and grinding food (Wharton, 1986). The lumen of the cuticle in nematodes with pharyngeal bulbs is corrugated to form three flaps or plates that assist in grinding (Lee, 1965).

The pharynx, which is a muscular, multinucleated, glandular pumping organ not separated into individual cells, has regions that are swollen to form muscular bulbs (Lee, 1965). The lumen of the pharynx is divided into five principal regions before it reaches the pharyngeal intestinal valve, which leads to the intestine (White, 1988). The first section is the buccal cavity, which is the small mouth-like area at the most anterior portion of the head (White, 1988). Following the buccal cavity lies the anterior procorpus, the bulb shaped metacorpus, a thinner and cylindrical isthmus, and finally the terminal bulb (White, 1988), also known as the posterior bulb (Lee, 1965). The pharynx has a total of 20 muscle cells, organized into 8 distinct layers, all having radial symmetry, except for the most posterior one located in the terminal bulb (White, 1988). These muscles are separated from the hypodermal and nervous tissues by hemidesmosomes connected to the basement membrane (White, 1988). Three of the eight are located in the procorpus, another three in the terminal bulb, one in the metacorpus and another in the isthmus (White, 1988). Radial muscles connect the collagen skeleton to the cuticle that lines the lumen of the esophagus (Wharton, 1986). Interior muscles and neurons are attached to this collagen skeleton (Brown, 2003).

The esophagus itself is composed of two concentric cylinders, which are connected by radial muscles (Wharton, 1988). This structure of radial musculature and cylindrical structures allows for more motility in diameter than in length (Wharton, 1988). Contraction of radial muscles causes an increase in pressure within the cylinder, decreasing the diameter and in effect, lengthening the area as well (Wharton, 1988). This only occurs if the pressure is less than the pseudocoel (Wharton 1988), otherwise referred to as the body cavity (Lee, 1965). The pseudocoelomic fluid is constantly under pressure, which allows the worm to eat, excrete, and relocate (Lee, 1965). Radial muscles contract until enough pressure can build up to increase the cylinder in diameter, growing shorter and wider (Wharton, 1988). This opens the lumen of the esophagus, allowing food to pass further down (Wharton, 1988).

In *Ascaris* nematodes, acetylcholine was applied to isolated regions of the body wall, causing the muscles of the worms to contract, relaxing when the drug was washed away (Lee, 1965). Nematode cells respond similarly to nicotine as they do acetylcholine (Lee, 1965). Piperazine is an anthelmintic drug that lowers the muscular response to acetylcholine, blocking the nerve-muscle junctions or the nerves (Lee, 1965). The worms are paralyzed, but will still

respond to electrical stimuli when bathed in piperazine (Lee, 1965). *C. elegans* body wall muscles become paralyzed when exposed to the drug levamisole, which is used to expel parasitic worms from host organisms (Culetto, E. et al, 2004). Levimisole is a nicotinic agonist which works by selective opening of ligand-gated ion channels, which are located in the membranes of muscles and nerves (Martin, R.J., Robertson, A.P., 2000)

We examined the effect of levamisole on the radial muscles by measuring their movement with and without the drug. I hypothesis that the levimiosle will indeed effectively anesthetize the specimen, leaving them paralyzed but alive. Inter-cellular movement should not be affected by this change, only muscular contractions and locomotion. Due to the fact that most processes, aside from locomotion, pharyngeal contractions, and anal dilations, are determined by turgor pressure, stopping muscle contraction within the specimen should not drastically affect cellular activity.

This paper describes a preliminary study, using only one worm for each condition to measure the effects of 10mM levamisole on pharyngeal contraction in *C. elegans*. Knowing how this affects the *Caenorhabditis elegans* can further research on the nematodes, which may have the possibility of affecting our knowledge of human processes along with methods to treat human pathologies, since some nematodes, though not *C. elegans*, are parasitic.

III. Materials and Methods

Materials:

- Ward's 'The 4 "L's" of *C. elegans* Lab Activity" kit and guide book
- Microwave (or boiling water, to heat the agar
- Colonies of Live *Caenorhabditis elegans* from Ward's and Carolina Biological Supply Company
- Bunsen burner
- Glass Pasteur Pipettes
- Rubber bulb
- Poly-Lysine
- Cover slips
- Tweezers
- Double Distilled H20
- Kimwipes
- Transfer Pipettes
- (-)-Tetramisole Hydrochloride solution, 10 mM (also referred to as Levamisole)
- 15 ml tubes
- Glass Microscope Slides
- Valap sealant (petroleum jelly (Vaseline), lanolin, paraffin; 1:1:1, w: w: w)
- Nikon Eclipse E400 Microscope
- Spot Insight QE Camera
- Nikon Eclipse E200 Microscope
- Sony Digital Interface DFW-x700 Camera
- Nikon SMZ660 Dissecting Microscope
- DAGE-MTI DC200 Camera
- BTV Pro Imaging Software
- Spot Imaging Software
- ImageJ 1.32j
- Adobe Photoshop 7.0

Methods:

Several cover slips were bathed in poly-lysine over night and rinsed with double distilled water immediately prior to use. Tweezers were used to hold the cover slips during the rinsing process. Slides were also rinsed with double-distilled water in order to clean them. The cover slips and slides were dry before mounting occurred. Chip chambers were created by placing small pieces of cover slip fragments around the perimeter of there the cover slip would be placed, using tweezers. Several drops of anesthetic (levamisole) were added via transfer pipette to the interior of the chip chambers, just enough to seal the cover slip without leakage. If there was additional liquid after the cover

slip was placed, a kimwipe was used to wick off the excess fluid. The levamisole was prepared using 8.46mL distilled water, a 15mL tube, and 24 mg of the concentrated powder, ordered from Sigma-Aldrich. The final concentration was around 10mM.

After several failures in the attempt to transfer worms from the plates to the slides, flame drawn Pasteur pipettes were made using a Bunsen burner. A bulb was placed at the top in order to achieve proper suction. Using a Nikon SMZ660 dissection microscope, *C. elegans* were located on the plates and drawn up using the flame-drawn pipettes. Any images taken were achieved by a DAGE-MTI DC200 camera. Using a transfer pipette, a small amount of levamisole was drawn up into the pipette and released in an effort to rinse the *C. elegans* out. This step was repeated several times. Cover slips were placed over the samples and valap sealant was added to the edges of the cover slip. Once the valap was dry, the slides were observed under a Nikon Eclipse E400 microscope. Images were taken using a Spot Insight QE camera. A Nikon Eclipse E200 microscope and a Sony Digital Interface DFW-x700 camera were also used to obtain data. The software used to capture the images included BTV Pro Imaging software and Spot Imaging software. Adobe Photoshop 7.0 was used to overlap images (for visual representation), add scale bars, and crop images.

Using ImageJ 1.32j software, the images of the specimen were measured from the tip of the anterior end (mouth) at the buccal cavity to the intestine, which lies beyond the posterior bulb of the pharynx. The furthest extension of the anterior end that was measured and considered to be 100% extended. The shortest contraction was then measured as a percentage of the entire anterior end, consisting of the alimentary canal prior to the intestine. This is easily distinguishable due to the darkened color of the intestine in comparison to the pharynx. This same technique was used for both anesthetized and unanesthetized worms, regardless of whether the full body was viewable or not. The speculation is that the full worm is not affected by the radial muscle contractions, so the length of the body as a whole should not affect the results.

IV. Results

The anesthetized *C. elegans* showed minimal range in the extent of contractions among the anterior portion. Figure 1 is an overlap image, containing the full extension of the *C. elegans* as a base image and the full contraction as the layered image. The only noticeable difference produced by this overlap is minimal blurring among the outline of the cuticle and the organs within the animal. No significant difference can be detected. Using ImageJ, the quantitative difference was measured for the anesthetized nematode. The difference was only .2365μm. The conversion from pixels to mm was determined by using photoshop to determine a scale.

A. 

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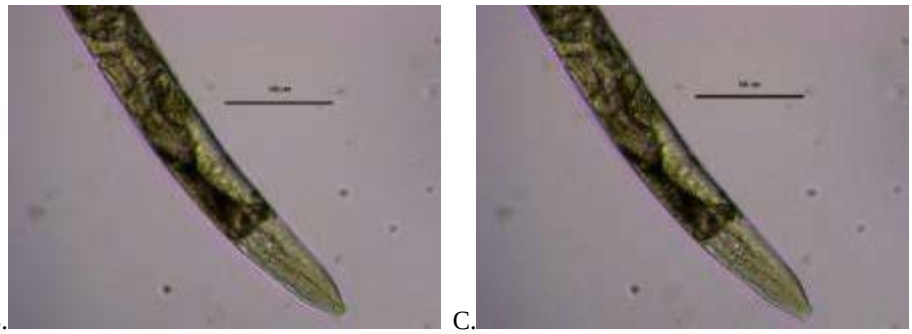

Figure 1: **A.** Overlapped images of a *C. elegans* anesthetized by 10% mM solution of (-)-Tetramisole Hydrochloride, at 0% and 100% extension. **B.** Full contraction of anesthetized *C. elegans* (99.79% extension): 111.401 mm **C.** 0% contraction of anesthetized *C. elegans* (100% extension): 111.6375mm.

Comparing contractile differences in the unanesthetized worm proved to be more dramatic. At full extension (100%), the anterior end measured to be 144.191 mm. At full contraction the anterior end measured 53.056 mm. This proves to be 36.796% of the full extension. Figure 2 images show this contractile difference.

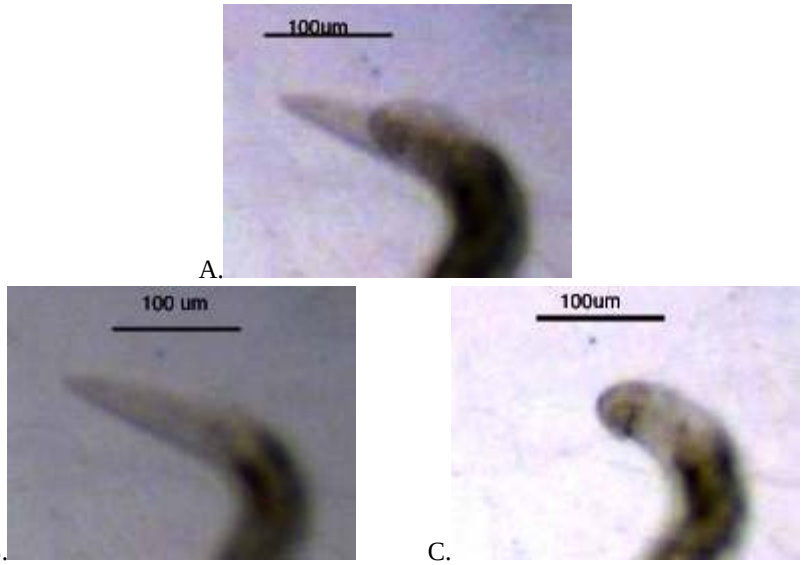

Figure 2: A. Overlap image of an unanesthetized *C. elegans* at full extension and at minimal extension.
B. Full extension (100%) of a *C. elegans*. [Base image for A] C. Full contraction for a *C. elegans* at 36.796% extension

Figure3.

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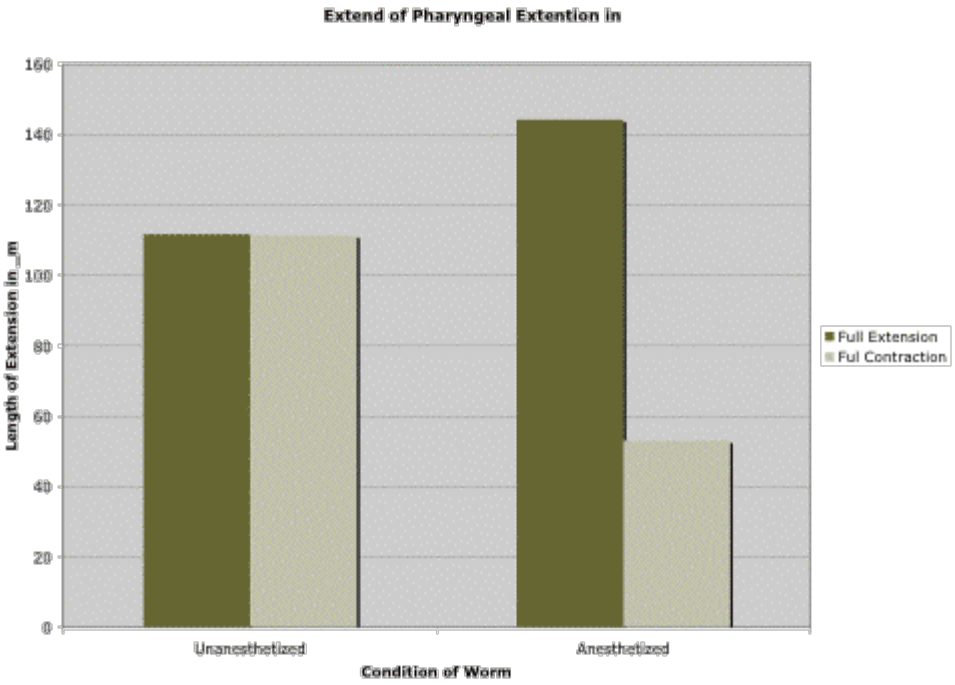

Figure 3 shows a graph comparing the length of extension for both anesthetized and unanesthetized worms. Fully extended means 100%.

V. Discussion and Conclusion

The data gathered suggest that Levamisole restricts muscular contraction in *C. elegans.* The nematodes are clearly alive, since some of the anesthetized worms twitch. Though, regardless of twitching, cellular behaviors can be observed within the worm. These data support the hypothesis that levamisole will restrict muscular movement, but will not kill the worms, nor affect other cellular activities. It is clear when we observe the chart that the percent of the pharynx that contracts in the unanesthetized worm is almost three times that of the anesthetized worm treated with levamisole. Levamisole is clearly an effective anesthetic.

I speculate that the neurons of the radial muscles (or possible just the muscles themselves) that control pharyngeal contraction indeed have receptors for levamisole. These are most likely the same receptors as acetylcholine and nicotine, known as the nicotinic acetylcholine receptor (nAChR) (Culetto, E. et al, 2004), which both affect muscle contraction in nematodes (Lee, 1965). When these receptors bind to the Levamisole, they are inhibited from contracting.

The levamisole must be similar in chemical formation to mimic acetylcholine and bind to its receptors. When these receptors bind to levamisole, it causes the muscles to be unable to contract. When acetylcholine is used instead, the muscles contract. One possible explanation to why levamisole disallows contraction could be that it is more similar to piperazine than acetylcholine. Piperazine lowers the body's response to acetylcholine, which causes muscle contraction. Piperazine disallows muscle contraction, paralyzing the worm. This is similar to how the *C. elegans* respond to levamisole.

To continue on with the thought the piperazine is similar to levamisole, experiments could be completed comparing control worms without anesthetic to worms treated with different concentrations of levamisole and different concentrations of piperazine.

There exists a possibility that the anesthetized worms were not attempting to contract these muscles, or that neither worm was measured in its full potential for extension or contraction. It seems only logical, however, that the worms are anesthetized since they do not swim after being treated with the drug. This shows us that locomotion is absent, which suggests that at least some muscles are being affected by the anesthetic. As far as the potential for extent of contraction or elongation, only more tests will determine this.

Though accurate data was achievable through the methods used, it might be more beneficial in the future to use the same magnification for every image. This problem was presented due to the fact that the unanesthetized worms move quickly and seemingly randomly throughout the slide, disallowing the ability to definitely predict where they would be located. For this reason, a lower magnification had to be used, disallowing for equal detail to be observed in each.

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IV. References

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