IN VITRO SYMPATHETIC CHICK NEURONS SHOW INCREASE IN AXONAL LENGTH AND A DECREASE IN FILOPODIA LENGTH AFTER 30 MINUTE EXPOSURE TO MERCURY

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Neurobiology Final Report
Bio 324 / Neurobiology
Wheaton College, Norton, Massachusetts, USA
April 24th, 2013

INTRODUCTION

Mercury is a neurotoxicant that is known for having dire neurological effects on the Central Nervous System (Leong, 2001). These neurological negative effects occur at a molecular level mainly to the microtubules and actin found in both axons and filopodia in neurons (Leong, 2001). Research has shown that Mercury disintegrates microtubules and actin (Leong, 2001) and both components are vital for the transportation of neurotransmitters along an axon (Kandel, 2013). Additionally, microtubules are necessary for axonal growth and movements of growth cones. Filopodia, which are found in growth cones, are actin-rich, thin spikes that are very important for sensing signals from the outside environment (Kandel, 2013). Axons and filopodia were studied in this experiment since both microtubules and actin are found in these components that are mostly affected by mercury element (Leong, 2001). Reasons for also having performed this experiment stemmed from the mercury spill that occurred in an underprivileged city in Peru where the mining company lied to the residents and told them that their neurological problems were not due to the mercury spill when in fact it was (Cabellos, 2003) and this research hopes to be of additional evidence of the negative neurological effects of mercury. Sympathetic chick neurons and dorsal root ganglions were used as the cells of study in this experiment. The cells were dissected from 10-day old chick-embryos (Gallus gallus) and were incubated at a temperature of 37 degrees Celsius after being titrated. This experiment looked at the effects of mercury on axonal and filopodia length. Additionally, this experiment tried to determine if there was a relationship with axonal length and filopodia length after exposure to mercury. It was hypothesized that there would be a decrease in length for both filopodia and axon when 100 nM concentration of mercuric chloride buffer is added to the live cells. Time-phase microscopy images were then used to compare before and after exposure to mercury to gather data to support the hypothesis.

MATERIALS AND METHODS

The experiment took a total of four weeks to perform. The first three weeks consisted of live cell studies and in the fourth week data was collected and analyzed.

Dissection

Procedures for dissecting the 10 day-old chick neurons were derived from a lab provided by Professor Robert Morris as well as Instructions for creating the float chambers (Morris, Primary Culture of Chick Embryonic Peripheral Neurons: DISSECTION, 2013).

Control and Experimental Neurons Set Up

The second week of the experiment, the sympathetic neurons were observed under microscopes and introduced to the desired perturbation: 100 nM dosage of mercury in the form of $HgCl_2$ that was dissolved in Tyrode's salt solution. The following steps were made to follow through with the procedure that was just shortly described.

To start, (2) chip chambers were created with chips (Primary Culture of Chick Embryonic Peripheral Neurons 2: OBSERVATIONS of LIVE UNBLABLED CELLS). Once both chambers were ready, one conver slip was labeled control, which had Tyrodes solution and no mercury and the other chamber was labeled mercury which contained both the 100 nM concentration of mercury with the Tyrodes salt solution.

The cell chamber that was labeled control was studied under a C92 Nikon Eclipse E200 microscope using a 10x objective lense with tungsten illumination for a period of 30 minutes in a Scorpio Mac Computer at the ICUC. Pictures were taken every 2.5 minutes using a Sony DFW-X700 on a 1.0x camera mount. Pictures gathered where using BTV software 6.0 and were then put together as a a time-lapse phase microscopy movie.

The second cell chamber with was then placed under a C92 Nikon Eclipse E200 Microscope. The 100 nM concentration of mercury was then introduced to the open side of the chamber by using a sterile pipette. Immediately afterwards the mercury concentration was added, group members started gathering data for a period of 30 minutes. A Scorpio Mac Computer at the ICUC was also used, and pictures were also taken every 2.5 minutes using a Sony DFW-X700 on a 1.0 camera mount. Additionally, the pictures were also gathered using BTV software 6.0 and the pictured gathered within the 30 minute period were also put together as a time-lapse phase microscopy movie.

For both control and experimental cell chambers, the temperature was monitored and kept constant at 37 degrees Celcius with an air heater (Morris, Primary Culture of Chick Embryonic Peripheral Neurons 3: DYING and

OBSERVING LIVE CELLS, 2013).

Control and Experimental Data Analysis and Quantification

Image J Software was used to measure axon length and diameter as well as filopodia length and diameter. The Filopodias were distinguishable and clear in the images if they looked like thin long spike projections that extended from growth cones (Kandel, Eric R. (2013) and those that carried these characteristics were used to gather data. The axons were distinguishable and clear in the images if they looked like a long thin projection of nerve cells that are about one micrometer in length and extend of from the soma body of a neuron (Kandel, 2013) and those that carried these characteristics were also used to gather data for the experiment.

RESULTS

Control and experimental data were collected for this experiment. Both axon length and filopodia length were measured using Image J for the control and experimental condition. Units used to measure length and diameter of axon and filopodia were pixels. Results demonstrate that there is a noticeable difference in length of axon and length of filopodia when mercury is introduced. Mercury decreased the length of filopodia while slightly increased, instead of the expected decrease, axonal length. In other words, the experimental test data demonstrate that filopodia length decreased to 0 pixels after being introduced to mercury. Axonal length had an unexpected increase in length when mercury was added.

When axon (red+mercury) from Graph 1 is compared to filopodia (red+mercury) in Graph 2, a difference in result is noticed: axon (red+mercury) from Graph 1 increased in axon length as minutes increased while filopodia (red+mercury) in Graph 2 decreased in length as minutes increased. This suggests that axonal length and filopodia length could be inversely proportional to each other, with or without a perturbation. In other words, length of axon and length of filopodia will be different or opposite in value when minutes increases and when introduced to a perturbation (+mercury) and when it is not introduced to any mercury.

When Filopodia control (blue) from Graph 2 is compared to Axon control (blue) from Graph 1 a difference in result is noticed: Filopodia control (blue) length in pixels decreased in length while Axonal control (blue) from Graph 1 in pixels length increased as time went by. This again suggests that axonal length and filopodia length could be inversely proportional to each other, with or without a perturbation. In other words, length of axon and length of filopodia will be different or opposite in value when minutes increases and when introduced to a perturbation (+mercury) and when it is not introduced to any mercury.

Figure A Filopodia Control

Filopodia are present at 30 minutes. The area where filopodia appear is boxed in the figure. Filopodia are the thin long spikes projecting away from the growth cone.

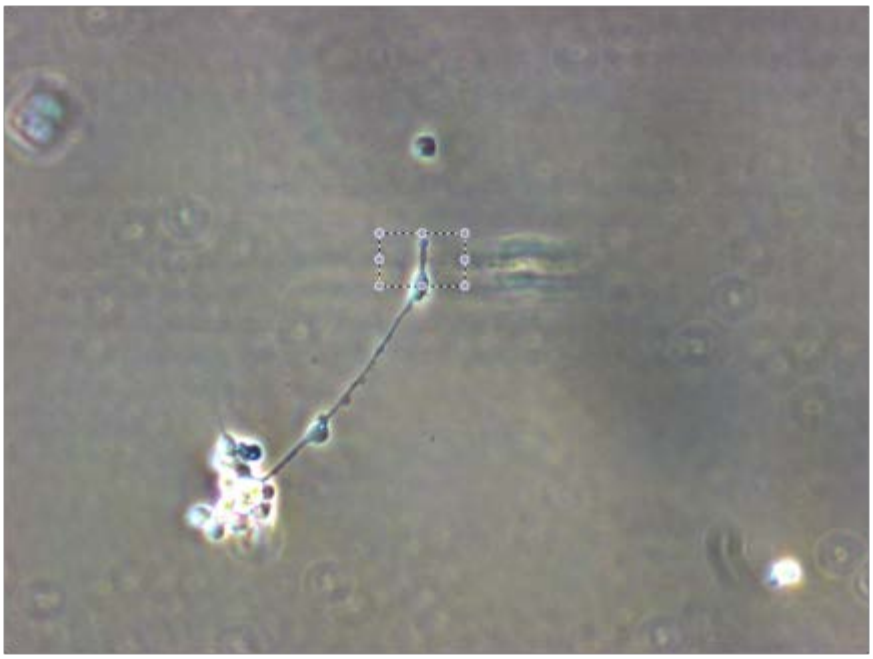
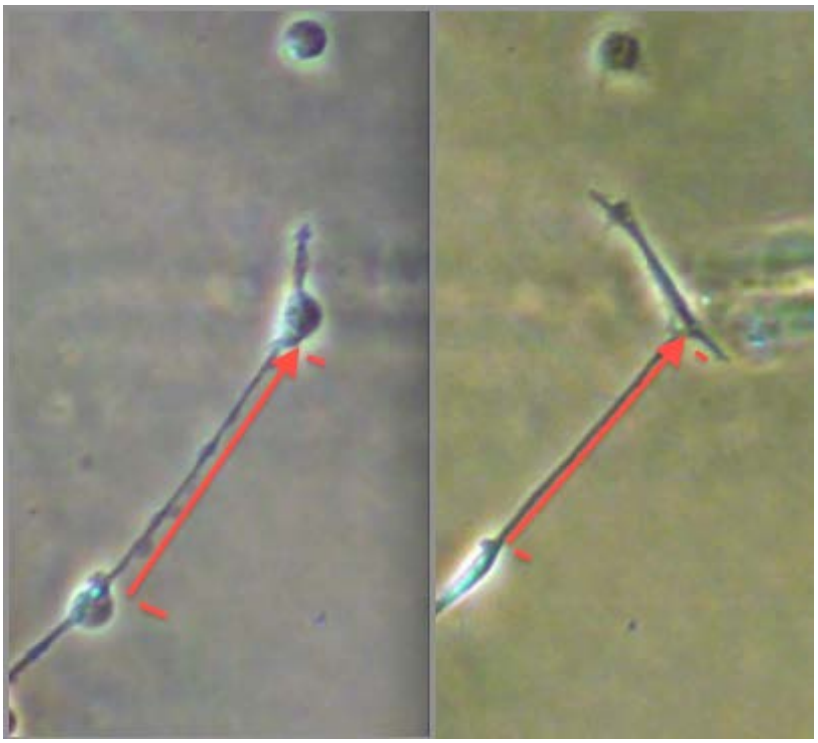


Figure B Experimental Filopodia

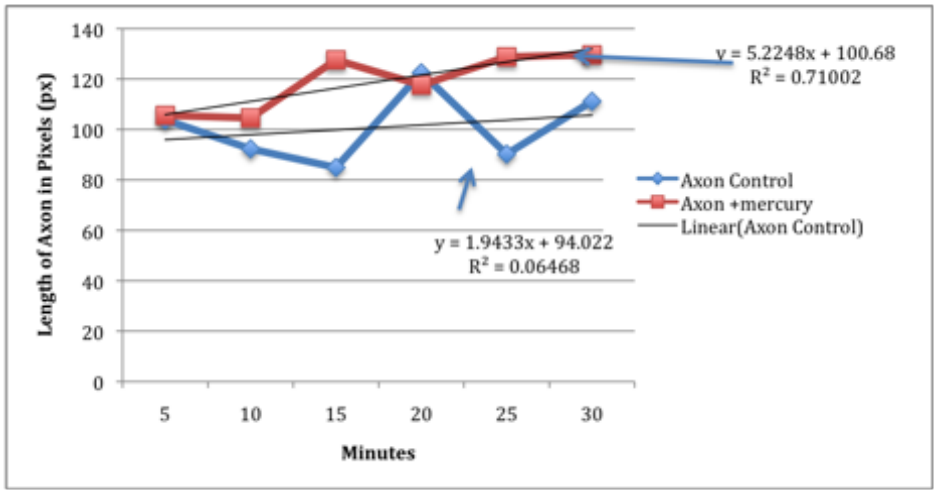
Figure shows no filopodia present at 30 minutes after mercury introduction. The area where filopodia were meant to appear is boxed in the figure.



A) B)

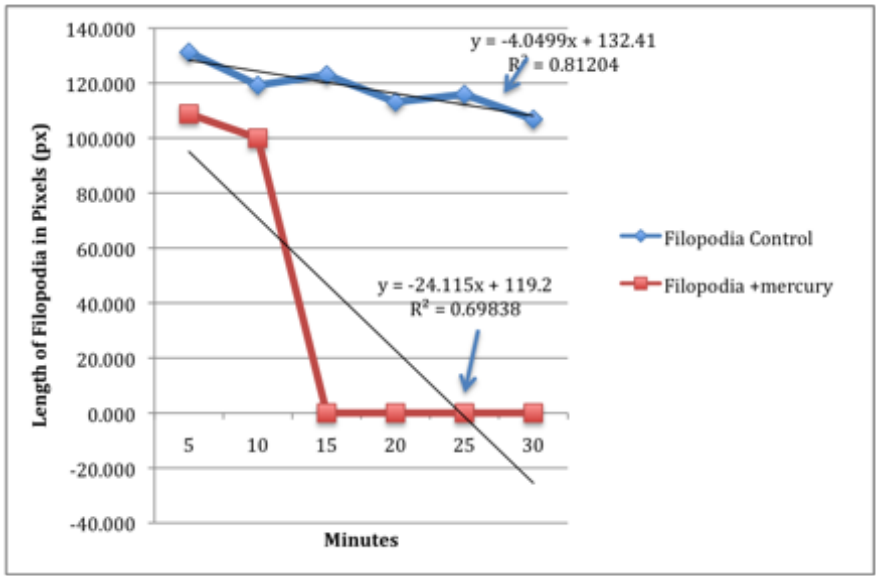
Figure C Experimental Axonal Increase 100 nM

Image A) shows axon increase at 30 minutes after exposure to perturbation. Image B) shows the same axon at 1 minute after exposure to perturbation. The length of axon in image A) is bigger in length and diameter than the same axon in image B).



Graph 1 Axonal Length for Control (blue) and Experimental (red) N=1

Axon length for control and experimental are similar in pixel value. They both have a positive trendline which indicates that there was an increase in length as the minutes progressed.



Graph 2: Filopodia Length for Control (blue) and Experimental Filopodia (+mercury) N=1

Filopodia length in pixels decreased as minutes progressed for both control and for the experimental filopodia with mercury.

DISCUSSION

The results do not support my hypothesis which stated that there will be a decrease in length for both filopodia and axons when 100 nM concentration of mercury was introduced to the live cells. In other words, length of filopodia and axons would show similar decreasing changes when introduced to any perturbation or with no perturbation at all. Quite the contrary, the data demonstrated that when a perturbation was introduced to the cells the axon length slightly increased and the filopia length instead decreased in length. Similarly, when no perturbation was added the axon length increased and filopodia length decreased as time passed. This observation suggests that filopodia and axon length do not show similar decreasing morphological changes. Additionally, the data collected also suggests that filopodia and axon growth are not directly proportional but rather could be inversely proportional.

One possible reason that axon length increased instead of decreased is that the axon may have been in the consolidation stage of axon growth (Lowery, 2009). During the consolidation stage, F-actin activity at growth cone decreases significantly which in turn causes the retraction of the filopodia found at the growth cone (Lowery, 2009). Since filopodias are attached to the growth cone, and the growth cone is attached to the end of the axon, it suggests that the retraction of the filopodia into the growth cone may cause a slight increase in length of the axon where said growth cone is attached (Fernandez, 2013). This could also be a possible cellular explanation to the results. To add on to the derived conclusion, perhaps buffer or mercury volume might have increased the axonal length as time passed. If this experiment were repeated a numerous amounts of times in the future and the same data were derived, it will again be concluded that filopodia length decrease when axonal length increases, vice versa.

There were some errors that could have occurred while performing this experiment. Firstly, some of the images gathered were a bit blurry making it hard to identify filopodia since they are significantly smaller in size than axons. Secondly, only one trial was performed for this experiment which lowered the significance of the data gathered. For future experiments, more trials should be performed to have a larger pool to work with. Thirdly, not the different size of both cells could have misled the interpreting of the data. If given the chance to repeat this experiment in the future, it would be beneficial and interesting to study the relationship growth cones have with the axon seeing as growth cones act as bridges for the axon and filopodias. Studying the communication of the axon with the growth cone and respectively the relationship between filopodias and growth cone more extensively would also be a significant future experiment to study.

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I would like to thank Zachary Dunn, Brianna Ferri and Allison Rainville for helping gather the control and experimental pictures.