

# The Characterization of Neurite Recovery after Mercury using environmentally relevant exposure in Vitro

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## Introduction

One of the most extraordinary features of the nervous system is the ability of growing axons to find their way around a complex cellular embryonic network. This navigation is carried out by specialized and highly motile neuronal growth cones located at the tips of developing neurites (Spencer, E. G, Lukowiak K, and Syed I. N, 2000). Their cytoskeleton is made up of microtubules and actin. Microtubules are dynamic structures that undergo continual assembly and disassembly within the cell. Intact microtubules are needed for the axoplasmic transport of proteins, proper axon outgrowth, the locomotion of the growth cones, and mitochondria transport (Hollenback 1995).

Methylmercury (MeHg) is an organomercurial pollutant primarily found in the aquatic environment and has been indicated in the disruption of microtubule integrity. Previous experiments indicated that this occur by depolymerization or inhibition tubulin polymerization of cytoplasmic microtubules (Miura K, Koide N, Himeno S, Nakagawa I, and Imura, 1999). MeHg is one of the most toxic forms of mercury because it can cross the blood-brain and placental barriers. It persists in several species of fish that are a part of the human diet (Rand D. M, Dao C. J, and Calson A. T., 2009). In addition, humans can have long-term exposure to mercury via the vapor specie ( $Hg^0$ ) found dental amalgam tooth fillings (Lorscheider L.F, Vimy J. M and Summers O. A., 1995).

In previous studies it has been shown that when exposed to MeHg neurites retract (Leong C. W. C, Syed I. N, and Lorscheider F.L., 2000).. In this study, I will investigate the effects of environmentally relevant amount of mercury on chicken, *Gallus gallus* embryonic neurons. I hypothesize that mercury will negatively affect the growth of neurites and that neurites will recover after exposure to mercury. The negative affects can be described as retraction of the neurites (shrinkage in length) and as the inability to grow by elongation. The recovery of neurites is described as the re-growing of neurites (elongation).

## Materials and methods

### Primary culture of chick embryonic peripheral neurons

Chick sympathetic chains and dorsal root ganglions (DRGs) were dissected and cultured as done in Morris, R.L. (2011a). The embryos that were dissected were at least 10 days old. In addition to the materials used in Morris, R.L. (2011a) a Nikon SMz660 dissection microscope was used to dissect embryos. Dorsal root ganglia (DRGs) were incubated at 37°C overnight night before observations. First DRGs were incubated with 50ng/ml Nerve growth factor and 100Umg/ml pen/strep; the DRGs were then incubated with 100ng/ml Nerve growth factor (NGF) and 200Umg/ml pen/strep to observe if this increased in the concentration positively affects neurites growth. It was later discovered that the increased in concentration did not made a large difference and therefore the original concentration of NGF and pen/strep were used for future cell cultures.

### Observation of Neurons

The DRGs were plated out and growth dynamics of neuron were observed as done in Morris, R.L. (2011b). The observations were done with a Nikon Eclipse E200 microscope with phase contrast. The camera used was a Sony DFW-x700 with a 1.0X C-mount, using BTV image software version 6.0b1 installed on an I-Mac computer with Mac-OSX.

### Experimental procedure for mercury exposure

The mercury used for this experiment was 100nM HgCl<sub>2</sub> in HBSS, which was made up from a 10ml of 10μM HgCl<sub>2</sub> in 0.05% HCl. The cells were mounted on the microscope and then heated to 37° with a Lasko ceramic air heater model 754200 for observation. The cells were observed for six minutes prior to mercury exposure. Pictures of the axons were taken every two minutes for the duration of the six minutes. The growth medium was then replaced with 0.7 ml of 100 nM HgCl<sub>2</sub> in HBSS via a flow chamber; the cells were then exposed to mercury for 30 minutes. At the 30<sup>th</sup> minute a picture of the axons were taken. The mercury was then washed off and replaced with growth medium after the 30

minutes duration. The axons were then observed for an additional 30 minutes, this was done to allow enough time to observe if recovery would occur or not. Again at the 30<sup>th</sup> minute pictures were taken of the axons. The initial six minutes observation was done to ensure that the neurons were capable of growing. A growth rate of approximately 7 pixels/minute was calculated for the experimental neurons, this was also done for the control.

## Analysis

To test the effects of mercury on the axons, the length that the axons grew were calculated before, during and after mercury exposure using Image J software, version 1.45b available on the I-Mac computers in the ICUC lab at Wheaton College, Norton MA. In order to control for the flow of mercury, a control with growth medium was done prior to the mercury experiment. The average length of the axons were calculated from pictures taken before, during and after for both the control and experimental neurons. The brightness and contrast of the images were not adjusted. In this study the length of axons were measured and compared to determine the effects of the mercury exposure, in order to complete this, these following steps were done:

A specific spot was identified before the axon length was measured; in this case, dirt in the same focal-plane that did not move throughout the experiment was used as a starting point for measurements.

Then using the segmented line tool in the image J software, a line was drawn from the dirt following the axon all the way to the axon tip (the line was drawn on the axon).

This was done for all the images taken before mercury exposure, the images taken during exposure and the images taken after mercury exposure.

The lengths of the lines were then calculated in pixels using the measuring function on the Image J software.

After the lines were measured the average length of the axons (before, during and after) was calculated.

The average length for the axon before, during and after mercury exposure were then graphed.

## **Results**

In this current experiment when the neurons were exposed to mercury the neurites did not retract (shrink), and appeared to be stationary. The neurites did not grow, during the first six minutes when the cells were mounted the average length was calculated average to be 367.023 pixels, during the 30 minutes exposure to mercury the average length of the axons measure in this experiment was calculated to be 411.343, the average length only increased by 44.32 pixels. After the mercury was washed off and the cells were given a small amount of time before observations were recorded, the axons were observed growing again. The average length for the axons after recovery (re-growth by elongation) was calculated to be 626.42pixels, this represented a 58.56% increase in the length of the axon after the mercury was removed and the neurons were allowed to recovery. The average length in pixels is represented in **figure-3b** for the mercury experiment.

**A**



**B**



**C**



**Figure 1:** Control phase images of axon, image (A) is an image of an axon before buffer flow; image (B) is an image of

an axon in the new buffer (C) is an image of the same axon when the buffer was removed after 30 minutes.

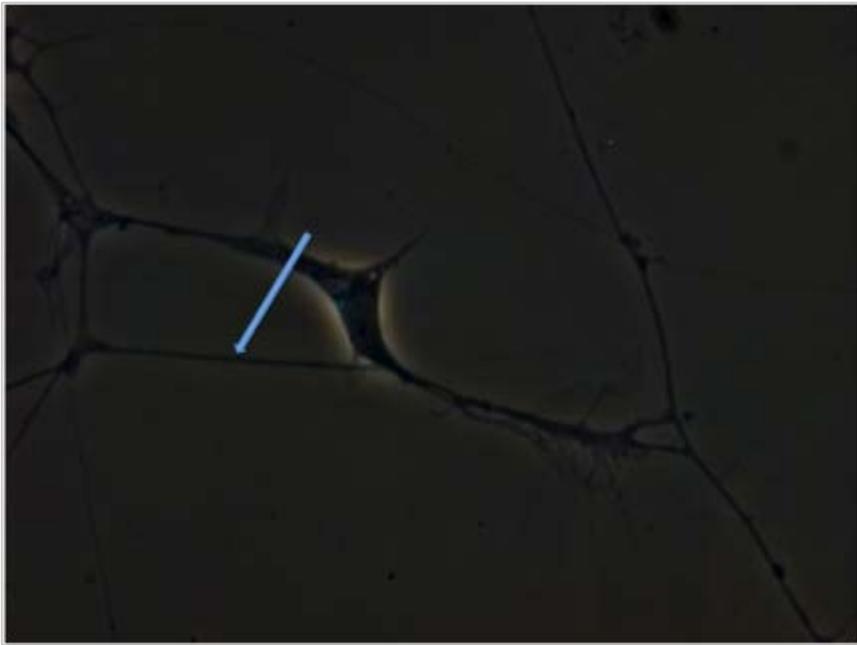
**A**



**B**

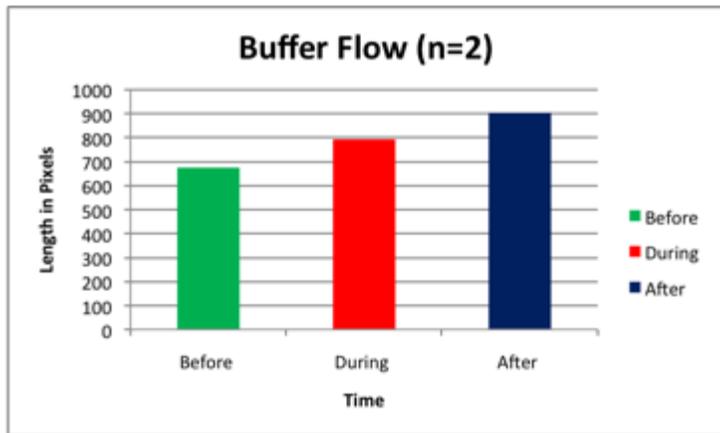


**C**

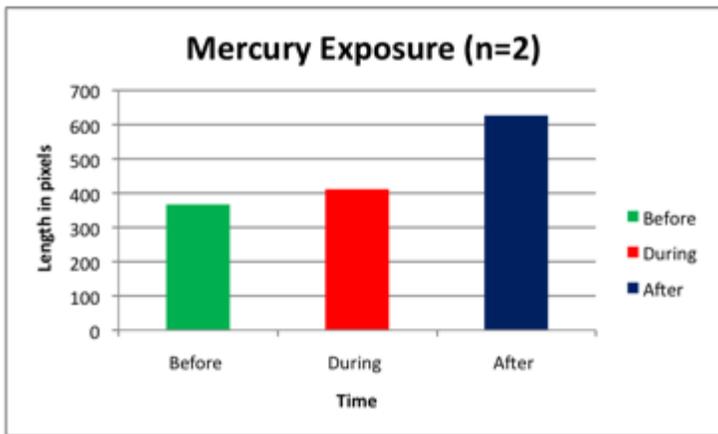


**Figure 2:** Phase image of experimental axon, image (A) is an image of growth cone before mercury exposure, image (B) is an image the same axon during mercury exposure and image (C) is an image of the same axon after mercury exposure.

A



B



**Figure 3:** Graph (A) shows the average length of neuron axon before, during and after the buffer flow, as you can see the growth of the axon increased by approximately 100 pixels from before to after buffer change. Graph (B) shows the average length of neuron axon before, during and after mercury exposure, as you can see the axons increased approximately 44 pixels in length, but from during mercury exposure to after mercury exposure the axons increased approximately 220 pixels in length.

## Discussion/Conclusion

The hypothesis was supported by the results in this current experiment. In previous research when neurons were exposed to mercury the neurites retracted by shrinking in length (Leong C. W. C, Syed I. N, and Lorscheider F.L., 2000). In this experiment, it was observed that the axons did not retract, but they did not grow very much either, this supports the hypothesis that mercury may inhibit axons growth.

The inability of the axons to grow can be attributed to the effects mercury on microtubules integrity (Miura K, Koide N, Himeno S, Nakagawa I, and Imura, 1999). The axons observed during this exposure did not grow possible because microtubules were unable to polymerize and since the cytoskeleton of an axon is made up of microtubules, the axon may not have elongated. If this was the case when the mercury was washed off and replaced with growth medium, the microtubules were perhaps again able to polymerize and therefore, the axon was observed growing again.

The findings of this study suggest that neurites does not always retract, in other words microtubules does not always depolymerize in the presence of mercury. If this was the case, this study contradicts the findings of previous research. These results raise new questions about the effects of mercury on neurons. The study was conducted with a small population of neuron (N=2), and even though the data collected suggest the mercury is capable negatively affecting the growth of axons by way of inhibition to grow to and not retraction. It is necessary to conduct further experiments in order to further investigate the effects and mechanism of mercury exposure.

I will refine this study but increasing the population of neurons in order to collect enough data to conduct statistical analysis to determine the significance of the data collected. The current data collected is limited, if more data is to be collected in future studies that support the current hypothesis, it will strengthen the conclusions made in this study.

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