# The Effects of Mercury on Axonal Growth with Implications for Alzheimer's Disease

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## Introduction

Effects of mercury cause severe brain damage in young children, adults and elderly people. Mercury comes in different forms; there is organic mercury ($Hg_2Cl_2$), inorganic mercury that are found as salts and elemental mercury is found as a liquid or quicksilver (Hock, 1997). Each form of mercury is found in a different place: organic mercury (methylmercury) is found in fish that consumed mercury by eating algae or by eating smaller fish that consumed algae; the methylmercury concentration increases has it goes up the food chain. The inorganic form of mercury is found in batteries or laboratories whereas the elemental form of mercury is found in glass thermometers and dental fillings (Hock, 1997). Different forms of mercury affect different parts of the neuron. It has been suggested that organic mercury affects the structure of the microtubules and inorganic mercury affects the surface membrane structure of the neuron (Miura, 2000). The addition of mercury to a healthy live neuron will affect the neuron immediately, affecting the growth of the axon (Miura, 2000). Axonal growth is extremely important to normal development and it requires many mechanisms to obtain the axonal elongation (Goldberg, 2003). The main cytoskeletal elements that are needed for axonal growth are microtubules and actin filaments (Goldberg, 2003). These properties hold the plasma membrane of the axon, so blebbing of the plasma membrane does not occur. Axonal growth takes place at the growth cone where lamellipodia and filopodia occur; growing the axon to its designated target or targets (Goldberg, 2003). As the microtubules are polymerizing, there are also neuronal growth factors in the peptide family called neurotrophins, which act as a signal to induce the axonal growth development (Goldberg, 2003).

The model system used in this experiment was *Gallus gallus* because their nervous system is not developed by the tenth day; not allowing them to feel pain. The sympathetic nerve chains and dorsal root ganglia were already formed which was dissected out. Also, their neurons are very similar to human neurons, having the same mechanisms and processes of the nervous system; allowing data of the effects of mercury to be test and relevant to humans (Miura, 2000). In the study, we treated chick sympathetic neurons with a concentration of a mercury solution to see the effects of mercury on axonal growth. I hypothesize that under different dosages of mercury axonal growth will slowly decrease to the point where the axon will retract back to the cell body, stopping the axonal growth (Hock, 1997) (Miura, 2000). The growth decreases due to the effects that mercury has on the microtubules by causing them to depolymerize (Tanaka, 1995). The references suggested that depolmerization of the microtubules decreases the axonal growth by causing the axon to bleb, breaking the interaction of axons and by the retraction of the axon towards the cell body (Charras, 2008). Increased mercury levels affect the assembly of the microtubules by inhibiting the protein ADP-ribosylation and also by inhibiting the protein kinase C (Mutter, 2010). Depolmerization of the microtubules, retraction and blebbing of the membrane decreases the axonal interaction by the decrease of the axonal growth. By inhibiting both of these proteins it causes neurofibrillary tangles (NFT) which is the trademark of Alzheimer's disease, which it has been linked too. (Mutter, 2010).

In this study, we tested the hypothesis that axonal growth, which was measured by the volume of the axon, will decrease in the presence of mercury.

## Methods and Materials

Materials

When the data were collected, the computer programs BTV 6.0B1 software (for the images) and Image J 1.46R were used to measure the diameter and length of the axon. The Neurons were treated with mercury solutions in three concentrations of 1nM, 10nM and 100nM. The mercury is $HgCl_2$ and was diluted with Tyrode's salt solution to make the concentrations. Those data were used to calculate the volume of the axons. All the data were collected in the Wheaton College ICUC (Imaging Center for Undergraduate Collaboration) on IMac computers with Mac OS X 7.1 software and the computers that were used were called Scorpio and Leo that have the Sony DWF-X700 with 1.0X camera mount on the Nikon Eclipse E200 microscopes using an magnification of 10X under the phase optics. The Scorpio computer was used to take the pictures and analyzes the data from the control and the concentration of 1nM mercury Neuron whereas the Leo computer was used for the concentration of 10nM and 100nM mercury Neurons. The data was collected and analyze at 10X and the unit of measurement that the data were collected in was pixels.

Methods

Experimental procedures were carried out as Professor Morris for the primary culture of chick embryonic peripheral neurons 2 (2013a) and the primary culture of chick embryonic peripheral neurons 1 (Morris, 2013b). The chip chamber used in this experiment created a flow chamber that had allowed the flow of mercury across the Neurons while the Neurons were on the microscope stage. The mercury was flowed through before the incubation period. Flow chamber was made for each concentration. The control flow chamber was put on the microscope stage first and a Neuron that had a long distinct axon was chosen. Heat was added to maintain the "incubating" temperature of 37 degrees Celsius. A picture was taken every 5 minutes of incubation until it was incubated for 30 minutes. That procedure was followed for the 3 mercury concentrations as well. The concentration of mercury of either 1nM, 10nM or 100nM was flowed through the flow chamber containing the Neurons. The procedure was done one time during one full lab period which was three hours.

Data analysis

The data were collected by measuring the length and width of the specific axon of each variable using the image J program and from that, the volume was calculated, assuming that the width was equal to the diameter. The length of the axon was measured by starting at the end attached to the cell body, which was seen by the distinct and abrupt change in color. The measurement started at the ease of the cell body to the farthest axonal growth cone. Each axon was measured initially, at 5, 10, 15, 20, 25 and 30 minutes of the incubation period. There were four different axons that were measured: one for the control, one for the 1nM concentration of mercury, one for the 10nM concentration of mercury and one for the 100nM concentration of mercury. The diameter was measured by taking the initial width of the axon and dividing that measurement by two to find the halfway point. Each time the diameter was measured, at all of the different time frames, the diameter was measured at the same half way measurement. The data of the length and diameter of the each axon was quantitated to determine the volume of the axon at all the different time points during the incubation period. It was quantitated by using the equation for volume which is volume = $\prod R^2$ X L. These were points assuming the axon is a perfect cylinder. In this experiment the control was Neurons growing without exposure to mercury but with buffer exchange only.

# Results

The control Neuron's axon was long throughout the observation time showing a lot of lamellipodia and filopodia at the growth cone (Figure 1.0). The Neuron that was treated with 100nM concentration of mercury showed blebbing and depolmerization of the axon (figure 2.0). The axon that was treated with a concentration of 1nM of mercury showed a drastic effect 5 minutes into the incubation after the mercury was added (Figure 3.0). There was a decrease in axonal volume, from 10 minutes to the end of incubation but the axon kept growing from the beginning to the end of incubation. Using the time-lapse methods, there was growth seen in the 1nM axon, showing a slight effect of mercury on axonal growth. Mercury effect was seen on the axon that was treated with the concentration of 10nM mercury. There was a decrease in the axonal growth, showing blebbing. The effect was notice in the first 5 minutes into incubation, where the axon blebbed (Figure 3.0). The blebbing was seen on the membrane of the axon and it is observed in the data that the axonal growth was decreasing. The axonal volume decreased over the period of incubating. The 100nM concentration of mercury was flowed through the flow chamber, the plasma membrane starting

blebbing extensively. It was observed that the axon fully retracted and the growth of the axon was inhibited (Figure 2.0). The axon that was treated with the concentration of 100nM mercury, decreased in volume by about 7 times its initial volume (Figure 3.0).

Figure 1.0: The control neuron of the experiment gave a baseline for the axonal growth during a 30 minute incubation period. This picture was taken at the end of incubation.

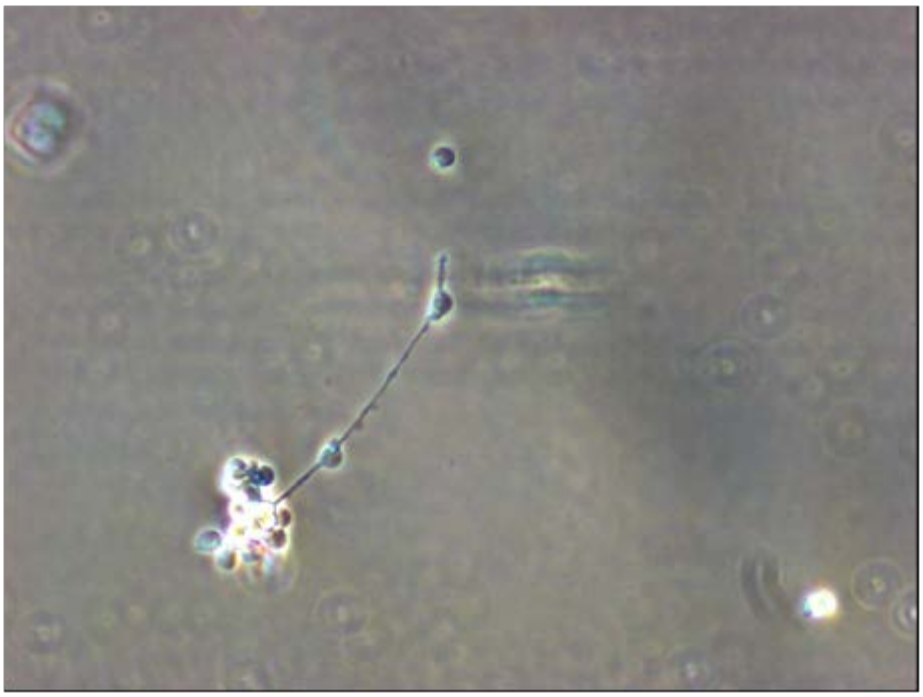

Figure 2.0: This experimental neuron was treated with the concentration of 100nm mercury. The picture was taken at the end of incubation and the blebbing of the plasma membrane and full retraction can be observed.

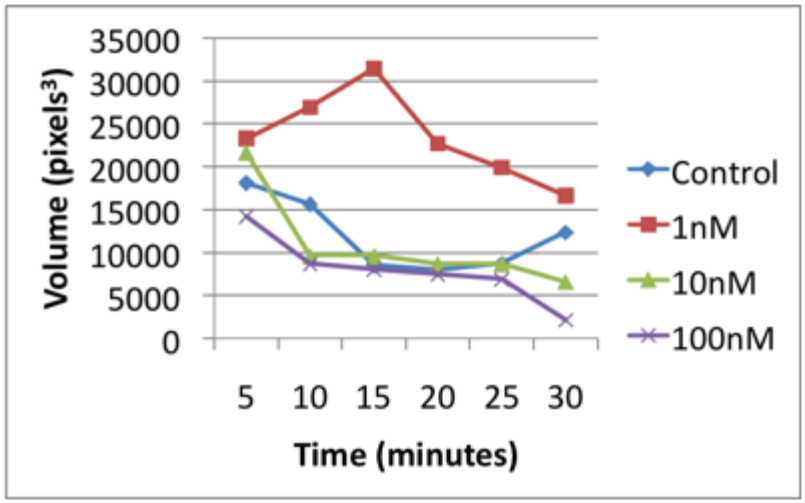


Figure 3.0: The volume of axonal growth was measured by the length and width of the axons, which were measured in pixels$^3$. It graphs the valves over the incubation period.

## Discussion and Conclusions

The current study showed that axonal growth decreased when in the presence of mercury. Our hypothesis is supported by the experiment that mercury negatively effects axon growth, decreasing the growth of the axon. In the three axons that were treated with 1nM, 10nM or 100nM concentration of mercury, all the axons showed a decrease in axonal volume. As the dosage of the concentration of mercury increased, the smaller the axonal volume became. The mercury targets the microtubules of the axon (Miura, 2000). The data suggest that the microtubules polymerize to increase the growth of the axon but in the presence of mercury, the microtubules structures are depolymerized (Mutter, 2010). The decrease in the axonal growth was seen by the retraction of the axon back to the cell body. The decrease was also observed by the depolmerization, swelling or blebbing of the plasma membrane (Charras, 2008). The data suggest that depolmerization of the microtubules happened more rapidly in the higher dosages of the concentration of mercury because of the easy ability for mercury to fuse through the plasma membrane into the neuron (Miura, 2000). The mercury does not just affect depolmerization but affects the subunits that make up the microtubules, which are tubulin; the presence of mercury inhibits the growth of the subunits (Miura, 2000).

With more data and multiple trials those data could lead to the cellular understanding of a neurological disease. Mercury effects the axonal growth, causing depolymerizing of the microtubules and inhibiting the growth of the subunits of the microtubules, which leads to neurofibrillary tangling (Mutter, 2010). Neurofibrillary tangling is a hallmark of Alzheimer's disease (Mutter, 2010). There is a link between mercury and Alzheimer's disease (AD) (Mutter, 2010). In Alzheimer's patients' brains, there is an increase of mercury content. In a recent study, 80% of the Alzheimer patients' had high level of mercury within the brain (Ely, 2001). The study found that in those patients with high levels, the mercury deactivates enzymes, for example the enzymes that are important for the production of the proteins that make up the subunits of the microtubules (Ely, 2001). Also, mercury causes the increased levels of αβ domain in the cerebral spinal fluid. αβ domain is a tertiary protein structure that has one domain that carry out important processes and mercury cleaves the αβ domain (Hook, 1997). The increased level of αβ domain is linked to Alzheimer patients, which these patients have a two-fold higher concentration of mercury within the brain than a non-Alzheimer patients (Hook, 1997).

During the current experiment, error would be reduced. When choosing an axon to manipulate with mercury, there was not a cell that had just one axon. The axons where only found in bundles, containing many axons, so the effect of mercury on just one axon could not be determined. To refine the experiment, it would be to use only one axon and watch the effects of mercury. That could be done by plating the neurons more sparsely on the coverslip. A higher magnification would be used to be able to see the cellular behaviors happening within the axon. Also, more trials would be conducted to gather more data to be able to observe and analyze the effects of mercury. Some future experiments that could be conduct to extend the data would be to use the amount of mercury that is found in our environment, like in fish, and use that amount over a longer period of time. Mercury concentration in fish are in categories from low to highest; highest being 0.5 parts per million which this food source should not be eaten and the low is 0.09 parts per

million which there is no limits on consumption (NRDC, 2013). That would allow the observation of the environmental concentration of mercury effects on the axon and would show the long term effects of the environmental concentrations on axonal growth. This is a different from the current experiment because it would be conduct over a longer period of time and it is in a concentration that is commonly found in a food source that is eaten by millions of people. Also, another idea would to observe if there was a protein that could act as an inhibitor to the mercury not allowing the mercury to inhibit the enzymes that are involved in important processes of the brain (Mutter, 2010). There are many directions the experiment can go because neurodegenerative diseases are becoming more common within the United States affecting many lives (Mutter, 2010).

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