Mercury Exposure Decreases the Extension Rate of Neuronal Filopodia

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INTRODUCTION

Neuronal filopodia are narrow (0.1- 0.3µm), hair-like structures filled with packed bundles of filamentous (F)-actin (Mattila & Lappalainen, 2008). Filopodia are known to be responsible for the cell's migration or extension. Filopodia are well fit to serve as antenna-like sensors, because they can extend to great lengths and cover large amounts of area to make sure the environment is safe enough for the cell to continue in that direction (Davenport et al., 1993). The elongation of the actin filaments pushes the edges of the cells forward, which promotes the cells movement in the direction the actin filaments are pushing on the cell. According to a study done by Mattila & Lappalainen in 2008, filopodia are also involved "wound healing, adhesion to the extracellular matrix, guidance towards chemoattractants, neuronal growth-cone pathfinding, and embryonic development." Filopodia are able to sense their environment for food and threats because they have receptors that can signal these extra cellular molecules to warn the cell of what they are (Mattila & Lappalainen, 2008). Depending on the type of environment the filopodia are in, determines the number of filopodia there are (Rehder & Kater, 1992). For example, if filopodia are in a safe environment, such as in F-plus growth medium, the filopodia become more plentiful, long, and in a fanlike position around the cell.

Mercury is a naturally occurring element that is found in air, water and soil. Mercury can either be elemental or metallic, organic or inorganic (DHEC, 2012). Methylmercury is the most common for of mercury. Methylmercury is highly toxic and one way humans are exposed are when then eat fish contaminated with mercury (DHEC, 2012). Any type of exposure to mercury can be hazardous to a person's health especially during the developmental stages in life (O'Reilly et al., 2010). Exposure to mercury during the prenatal period is hazardous to the child and has the potential to cause disorders such as "cerebral palsy, seizures, microcephaly, and mental retardation" (Alattia et al., 2011). Prenatal exposure to methylmercury can also effect one's "memory, attention, language, cognitive thinking, and fine motor and visual spatial skills" (EPA, 2012).

There are many studies on filopodia and numerous studies on mercury. We know the importance of filopodia to a neuron cell as well as the toxic effects of mercury on humans, adult, child, and even prenatal. However, what is not understood is how mercury effects individual cells in the human body, specifically the filopodia. Knowing that mercury causes harm to neuronal development and other body parts in humans, it is suggested that the process of mercury poisoning starts off in single cells before the effects become visible to the human eye. This lead to the hypothesis that short term mercury exposure will cause a decrease in extension rate of neuronal filopodia. In this study chick dorsal root ganglia and sympathetic nervous chains were harvested from 10 day old Gallus gallus chick embryos. The harvested cells were then placed on a slide and put under a microscope to view images of the cell. The control group which was just the harvested cells not treated with mercury and the experimental group was the same cells treated with 0.5mL of a 100nm solution of mercury ($HgCl_2$).To measure the results of the experiment, time-lapse video microscopy were used to take pictures of the cell for 10 minutes at 15 second intervals to allow for visual representation of filopodia behavior both treated and untreated with a dose of mercury.

MATERIALS AND METHODS

Dissection:

Dorsal root ganglia (DRG) and sympathetic nervous chains were harvested from the dissection of ten-day old Gallus gallus chick embryos (Morris, 2013-1). The DRGs and sympathetic nervous chains were then placed in petri dishes and then into the incubator to grow following Morris (2013-1). The materials used for the chick embryo dissection is found in Morris (2013-1)

Observations of cells using Video-Enhanced Phase Microscopy:

After about a week in the incubator at 37 , the collected cells were plated on a microscope slide, in a specific manner to create a flow chamber as described in Morris (2013-2).
Once the plating was complete, the flow chamber slide was brought over to the ICUC in the Mars Center for Science & Technology, at the Wheaton College campus in Norton MA., to be observed. In the ICUC, the cells were observed with a Nikon EFD-3 microscope on a Mac OSX Version 10.5.8 at 40x magnification and on phase 100,40. A Windmere heater was placed about 3-4 feet away from the microscope but still facing the slide to control the temperature. A thermometer was attached to the microscope stage as well. The cells were heated to 34 - 40 during the experimental observation. The images of the cells were taken using a DFW-x700 camera. The imaging software used was BTV version 6.0b1. Once the temperature around the slide reached 34 , an initial photograph was taken. Additional photographs were taken every 15 seconds over a ten minute time span. The control group, which consisted of cells untreated with mercury and only the medium they have been living in, was observed first. After the 10 minutes, the old medium was flowed out by using Tyrode's salt solution. Once the chamber was filled, the Tyrode's salt solution was flowed out with 0.5mL of a 100nm solution of $HgCl_2$ until the Tryode's salt was completely flowed through following Morris (2013-3).

Quantification of Data:

Data collection/analysis was done using the computer programs ImageJ Version 1.46r, and Microsoft Excel Version 14.2.2. For the experiment, a filopodium is described in the images as a hair like structure that is protruding from a growth cone. The rate of extension was measured by looking at consecutive pictures of the chosen filopodium (1 picture per minute). The first picture taken was used as the base measurement of the filopodium length, which can remain in the units of pixels. The measurement was determined by using the point tool in ImageJ and going over to the very tip of the filopodium to retrieve the x and y coordinates of that filopodium tip. For each image there after, the mouse would go to the original x,y coordinate and trace the rest of the filopodium to the tip using the segmented line tool in ImageJ and measured the difference from the tip of the filopodium to the base coordinates. The images used in this experiment were from minute 0-10. The average length of the filopodium was taken and analyzed for each the control group and the mercury treated experimental group. The control and experimental net change was analyzed by simply adding the number of pixels for each of the 10 pictures of the filopodium for each group and then the totals were compared. The control and experimental amount of overall change in filopodium extension over the ten minute interval was determined and compared. The calculations to find this change were to subtract the observed length, in pixels, for each minute and then each change was added together. The extension rate per minute was averaged from the total change in ten minutes divided by the ten minutes and then observations were compared.

RESULTS

Two sets of data separated into 3 different categories were collected. One se of data belonged to the control group, which consisted of the cells in the normal growth medium. The other set of data belonged to the experimental group, which consisted of the same cells as the control group but were treated with 0.5mL of a 100nm solution of mercury. Figures 1A and 1B show the filopodium measured at the beginning of the 10 - minute interval (1A) and the length of the filopodium at the end of the time span (1B). Figures 2A and 2B represent the length of the filopodium at the beginning when mercury was first added (2A) and the extension after being exposed in mercury for 10 minutes (2B). These results reveal that the control group had a greater rate of extension than the experimental group treated with mercury. Figure 3 reveals that the control group extends at a faster rate per minute than that of the experimental group. The net filopodium extension sum for the control group was 325.9 pixels, which was about twice as much as the experimental group, which was 160.4 pixels (Figure 4A). The amount of change of filopodium extension (Figure 4B) reveals that the control filopodium extended a total of 99.41 pixels compared to the experimental filopodium that negatively extended 6.46 pixels in 10 minutes.

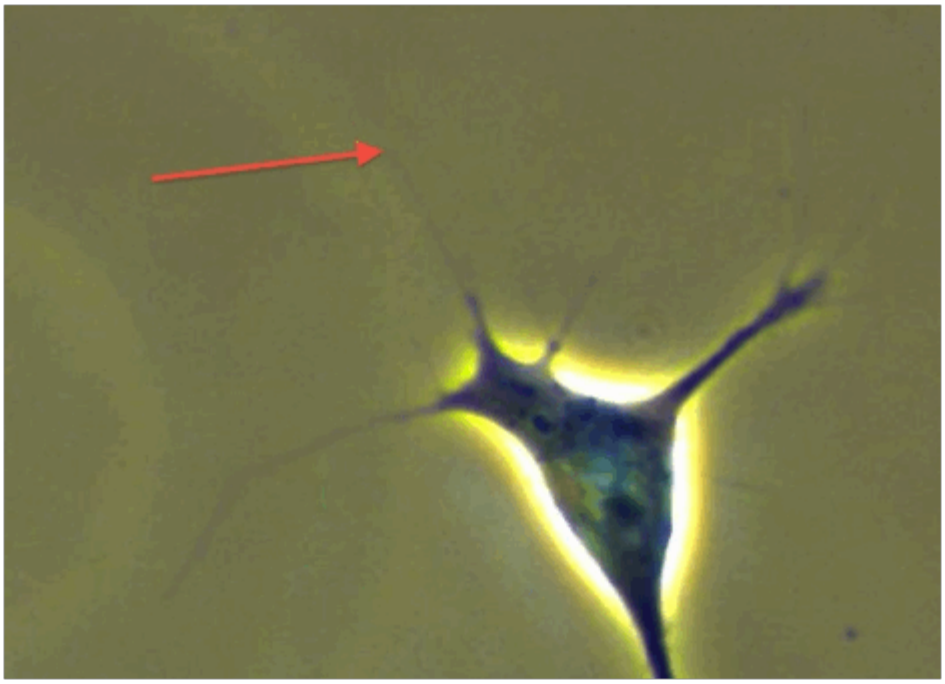

Figure 1A. An image of the very first picture that was taken of the control filopodium. The red arrow is pointing to the very tip of the filopodium to get the base coordinates (449,85).

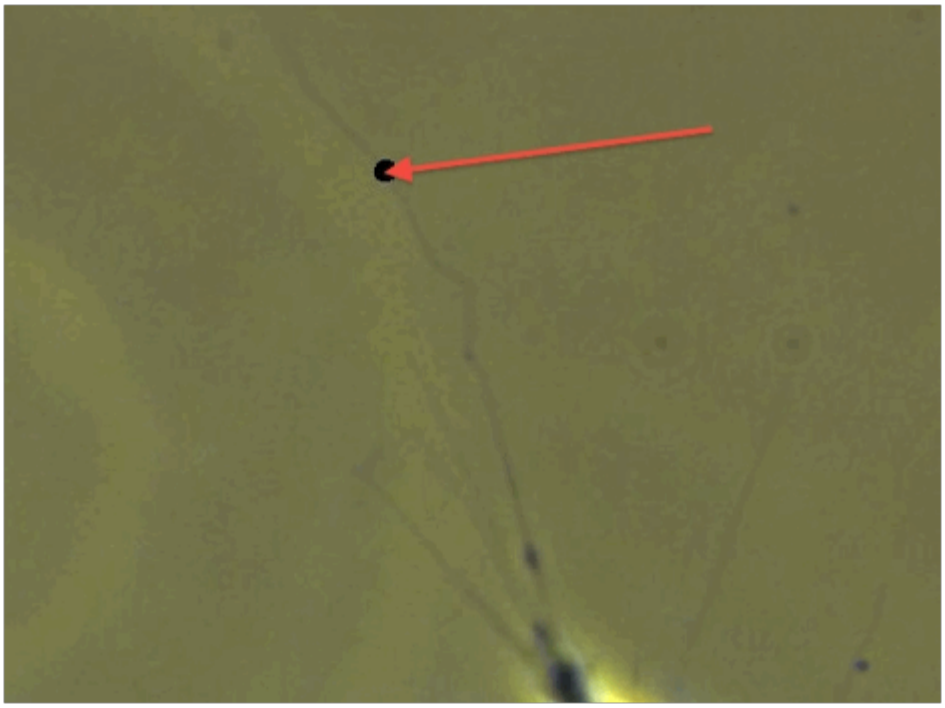

Figure 1B. An image of the last picture taken of the control filopodium. The red arrow pointing to the black dot is the original coordinates from figure 1A. Above the arrow is extra length of filopodium indicating extension. The extension after 10 minutes was 99.41 pixels.

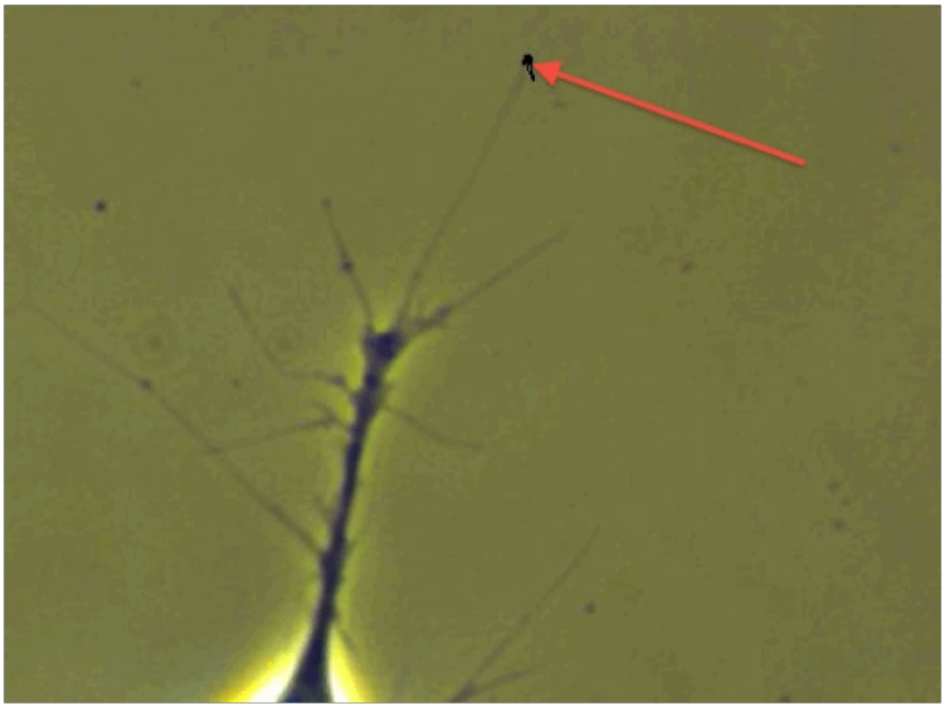

Figure 2A. An image of the first picture taken, of the same cell, just after mercury was flowed through its chamber. The red arrow is pointing to the black dot indicating the base coordinates where the filopodium will reveal how much it extended. The coordinates are (799,28).

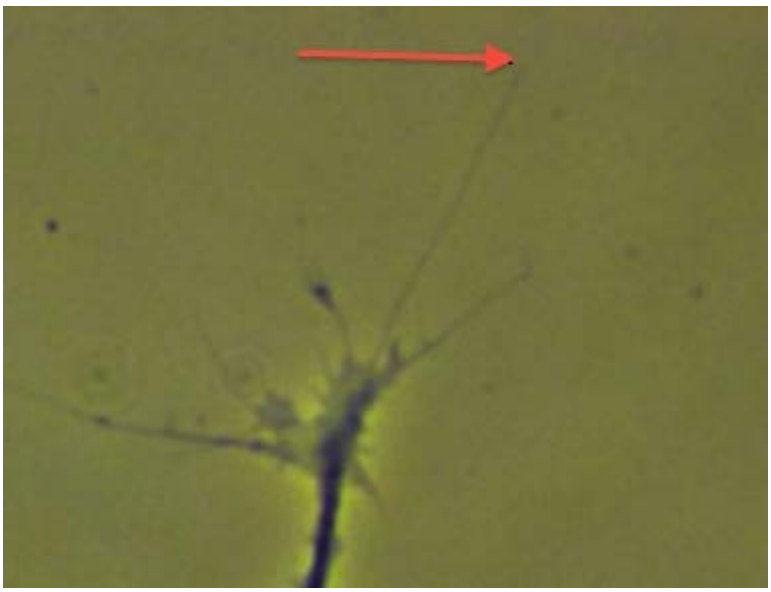

Figure 2B. Shows the last picture taken of the experiment filopodium. The red arrow is pointing to where the original coordinates are from figure 2a. There is a slight negative extension of 6 pixels after being exposed in mercury for 10 minutes.

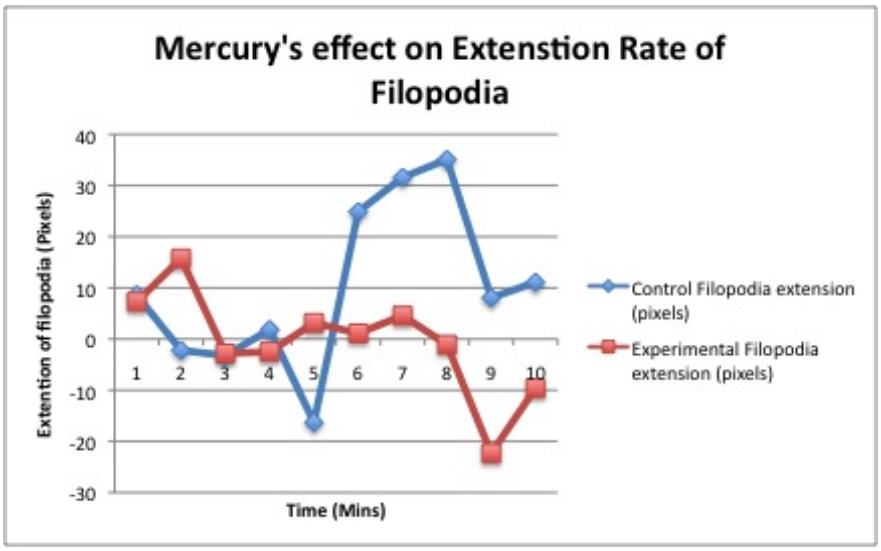

Figure 3. Shows how mercury effects the extension rate of filopodium. The blue line represents the extension rate of the control filopodium and the red line indicates the extension rate of experimental (mercury treated) filopodium .

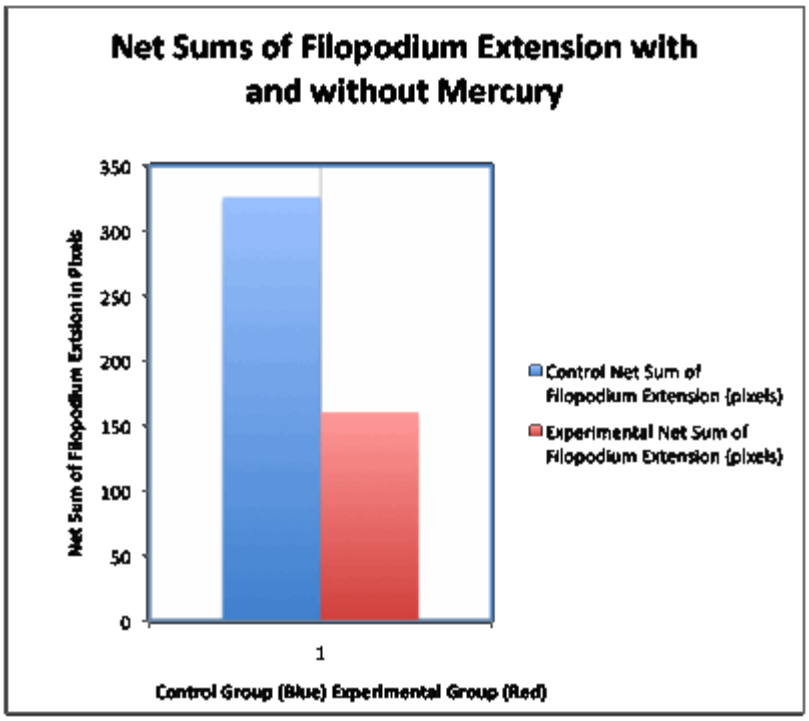

Figure 4A. Represents the net sums of filopodium extension with and without mercury. The blue column represents the untreated (control) group and how much extension the filopodium did over 10 minutes. The red column represents the mercury treated (experimental) filopodium and shows about half the extension in 10 minutes than the control group.

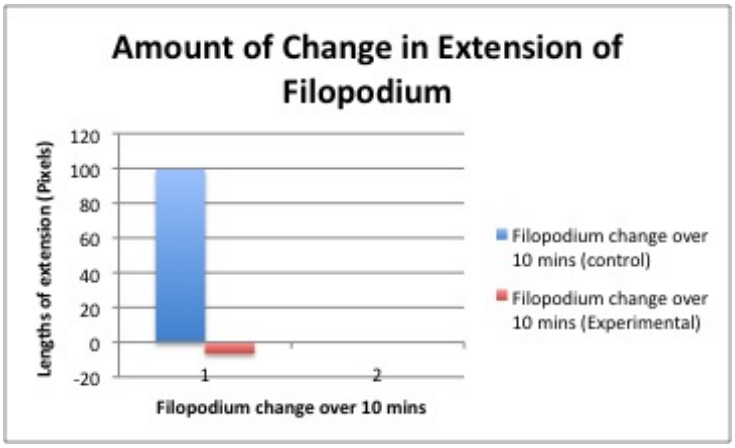

Figure 4B. Represents the amount of change in extension of filopodium over 10 minutes. The blue bar represents the control and the red indicates the extension of the mercury treated filopodium.

DISCUSSION

Based on the results of the experiment, the hypothesis was supported. Mercury does effect filopodia in a way that extension rates decrease once exposed to mercury. Overall, by the end of the 10 minutes the filopodium treated with mercury negatively extended by a total of 6 pixels and the filopodium not treated with mercury extended by a total of 99.4 pixels. The extension rate per minute for the control group was 9.94 pixels/minute while the experimental group extended at a rate of -0.65 pixels/minute. These results reveal that mercury does have an effect on filopodia in a way that slows their extension rate. Filopodia are driven by actin polymerization (Nemethova et al., 2008). This suggests that mercury may be having an effect on the actin filaments bundled in the filopodia. Since the rate of extension decreases when exposed to mercury, a possible reason for the slower extension rate could be that the actin polymerization activity is also decreased. Filopodia are also in close relationship with lamellipodial (Mogilner & Rubinstein, 2005**)**. The filaments that filopodia contain, are similar to that of lamellipodial in which they are polarized in the direction they want to move (Mogilner & Rubinstein, 2005). Filopodium mature from lamellipodial networks (Mogilner & Rubinstein, 2005), which brings the possibility that mercury is not only effecting the filopodia, but may be effecting other mechanisms that cause the filopodia to decrease in the presence of mercury.

The results provide further evidence from past studies what the functions filopodia serve to a cell. The filopodium examined show different behaviors in the two different environments they were placed in. The extension rates of the filopodium decreased in the presence of mercury as opposed to a healthy cell culture environment. A possible explanation for the results is in support of Davenport et al., 1993, that the filopodia act as a navigation system for some cells. They are acting as antennae to sense the environment around the cell. They sense mercury in the area and therefore they began to retract or slow down their extension to be more cautious to keep the cell safe. As opposed to the cell culture environment where the food is plentiful and the temperature is controlled to their ideal temperatures. The filopodia are healthy and they sense no threat in the environment. The actin filaments polymerize at a quicker rate allowing the filopodia to extend to greater lengths and promote cell movement.

If this experiment were to become reliable and valid, it must be repeated numerous amounts of times and come out with the same results. Also, further research would be made to search for reasons why these claims are true. For example, scientist may then want to find out what the filopodia are interconnected with because if the filopodia are effected by the mercury, most likely what the filopodia are made up of and interconnected with are affected as well. Filopodia are made of actin, which would suggest the actin is affected by mercury as well. A cell is like a machine. It is composed of many different parts that are all interconnected to one another and each piece of the machine has an important role to influence how the machine functions. If one part of the cell is affected by mercury, like filopodia are in this experiment, it can be assumed that other parts if not the entire cell is affected as well. If the experiment were to be repeated and reveal the same results, further research would be done not only on the filopodia by every part of the cell as well.

Sources of error or limitations in this experiment would be that only one specific filopodium was chosen to be measured through out the duration of the study. This is a limitation because there are questions that still develop to make these results difficult to be proven true. Suggestions for future research on how mercury effects filopodia,

researchers may want to study and measure more than just one filopodium in the cell. If you measure just the one filopodia, there may be questions left unanswered such as, do all filopodia on a cell react the same way when exposed to mercury? The only way to find that out is to test and measure the extension rate of each filopodia. Further research in this area of study, can reveal potential causes of the effects cells experience when exposed to mercury.With further research, more and more information will be revealed about how mercury effects a cell and how each system of a cell effects one another.

To refine this experiment, I would use different doses of mercury to see if there is some sort of threshold with how much mercury is fatal to filopodia. If more doses were added, would the increased doses effect filopodia more rapidly? If less mercury was added to the cell, would the filopodia experience any change in behavior? These are the questions that would be aimed to answer in the next experiment. I would also study more than one filopodium more like a specific area of filopodia to help the validity the conclusion that all filopodia act in the same manner when exposed to mercury. It also makes sense to take pictures for a longer period of time to see how longer exposure of mercury effects the cell. I think these adjustments would make the experiment more reliable and therefore valid.

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