The Effects of Mercuric Chloride on the growth of Neuron in Chick Nerve Cells

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Introduction:

Neuron like cells in all tissues have a cell body with a nucleus and other subcellular components that synthesize proteins, produce energy to fulfill the basic necessities of cell survival (Kandel, 2008). However, they are different than other cell types because of their essential function of transmitting signals through the nervous system (Knott. et. al, 2001). A neuron possesses an axon and dendrites that extend from the cell body and they are specialized for carrying electrical signals across the nervous system (Matus, 2001).

In this experiment, the effect of mercuric chloride on the growth of neuron in Chick embryo nerve cells was investigated. A large number of studies have been published on the difference in pathogenicity of mercury. Mercury is a ubiquitous environment toxin that causes a wide range of adverse health effects in human. Mercury poisoning is characterized by neurological disturbances (Guzzi et. al, 2008). There are three forms of mercury and each of them has its own profile of toxicity. Mercuric chloride has been used for many years in numerous products, including various medications, germicidal soaps, teething powders, and skin creams. There are studies suggested that mercuric chloride may induce fatigue, insomnia, weight loss, erythema, neuropsychiatry disorder (Guzzi et. al, 2008). For example, Mercuric Chloride when it is introduced to human body it is distributed to various organs, but it exerts toxic effects selectively on the brain. Mechanisms of how mercury affect the brain is not known, however, researchers believe mercury depresses cell proliferation (Guzzi et. al, 2008).

The effect of mercuric chloride ($HgCl_2$) on the growth of neuron was investigated at the cellular level by measuring distance in pixels via Image J1.40g from 10 day old chick embryo. In this experiment, brightness of selected neuron was used to generate the length of the neuron, and the length of the neuron was then used to calculate growth rate. This is an alternative way to measure growth rate indirectly. Chick embryo was chosen in this experiment due to its similarities to human nerve cells. I hypothesized that experimental neurons (from a point of selected on the axon to the tip of the neuritis) that are exposed to mercuric chloride will have a lower growth rate compare to neurons without exposing to mercuric chloride.

Materials and Methods:

Dissection:
Dissection method was used as described in "Morris (2011a)".

Cell Observation:
Cell observation method was used as described in "Morris (2011b)"

Cell staining and Observation:
Cell staining and observation method was used as described in "Morris (2011c)"

Experimental Procedure:

Control slide:

After staining chick nerve cell on to microscopy slide, neuron was observed via Nikon Eclipse E200 microscope under total 40X objective magnification. A picture was immediately taken at time 0 onto the slide via using BTV 6.0 b1. 5 minutes later, another picture of the neuron was taken. After the second picture 0.75ml of L-15 growth medium flow was applied to the slide for 5 minutes and a picture was taken again. At last, 5 minutes after L-15 growth medium was applied a picture was taken again. The whole procedure was operated under approximately 30^{o}C.

Experimental slide:

Same preparation and condition were used as the control slides. However, after the first 5minutes interval, 0.75ml of 100nM $HgCl_2$ flow was applied for 5 minutes. Immediately after that, L-15 growth medium flow was applied and 2 pictures were taken with 5 minutes interval between them. The procedure was operated under approximately 30^{o}C.

Data Analysis:

Images of control and experimental neuron slides were taken via BTV 6.0 b1 and quantified via Image J 1.40g using iMac OS X. A single point on the neuron was selected in the control and experimental slide and traced with segmented lines down the axon to the tip of the neurites and beyond the area of the axon (background) . Since the selected neuron has its own specific brightness compared to the background, therefore, the length of the neuron could be identified in pixels. The selected neuron was analyzed via brightness. Brightness value for this line was found via using plot profile under analysis tool in Image J. Brightness values was then turned into a histogram of gray value vs. distance traveled in pixels by Image J. Data from histogram was then exported into excel to obtain numerical values for the length of the selected neuron. The length of the neuron (distance traveled in pixels) was divided by time to generate growth rate values of the neuron (pixels/mins).

Results:

2 pictures of the control neuron slide and 2 pictures of the experimental neuron slide and were displayed in Figure1A -2B. The bottom neuron (highlighted in red) was selected for control and it was noticeable that the neuron has

experienced growth (figure 1A-1B) after L-15 growth medium flow was applied unlike experimental selected neuron (middle neuron) experienced less growth (figure 2A-2B) after 0.75 ml of 100nM mercuric chloride treatment was applied via observation.

A growth rate vs. time graph (figure 3) was constructed to demonstrate the growth rate of control and experimental selected neuron throughout 15 minutes. Growth rate values were obtained via length (distance in pixels traveled) over time, and length was obtained via brightness value. Figure 3 shows both control and experimental selected neuron experienced growth. However after mercuric treatment after 5 minutes, experimental selected neuron experienced more of a decrease in growth rate compared to control neuron by 3.3 pixels/mins at 10 minutes and 3.8 pixels/mins at 15minutes.

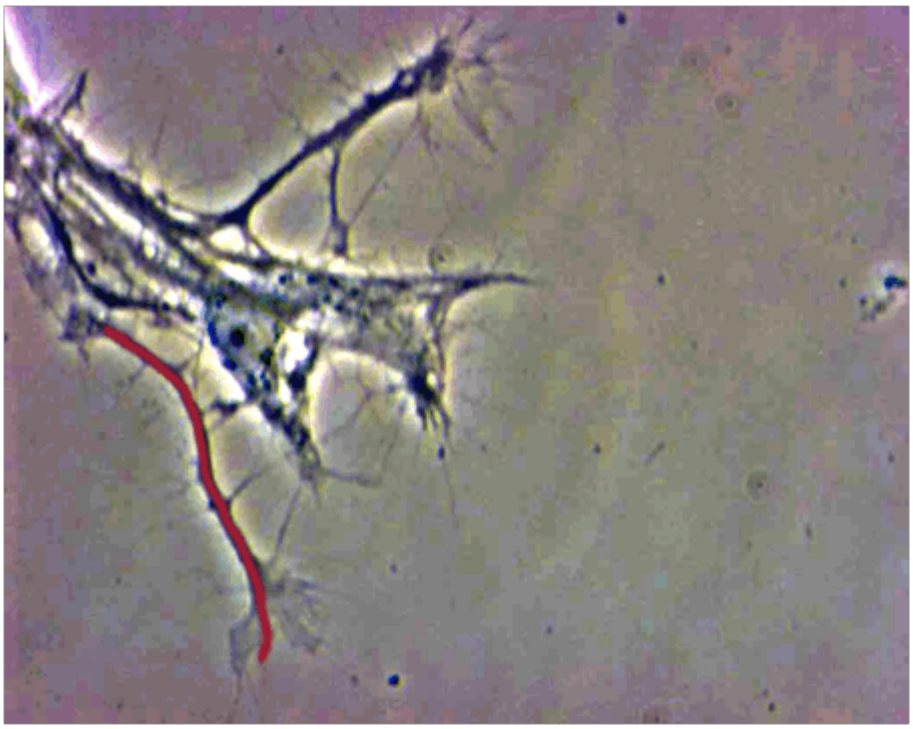

Figure 1A. Control neuron at time 0 under total magnification of 40X.

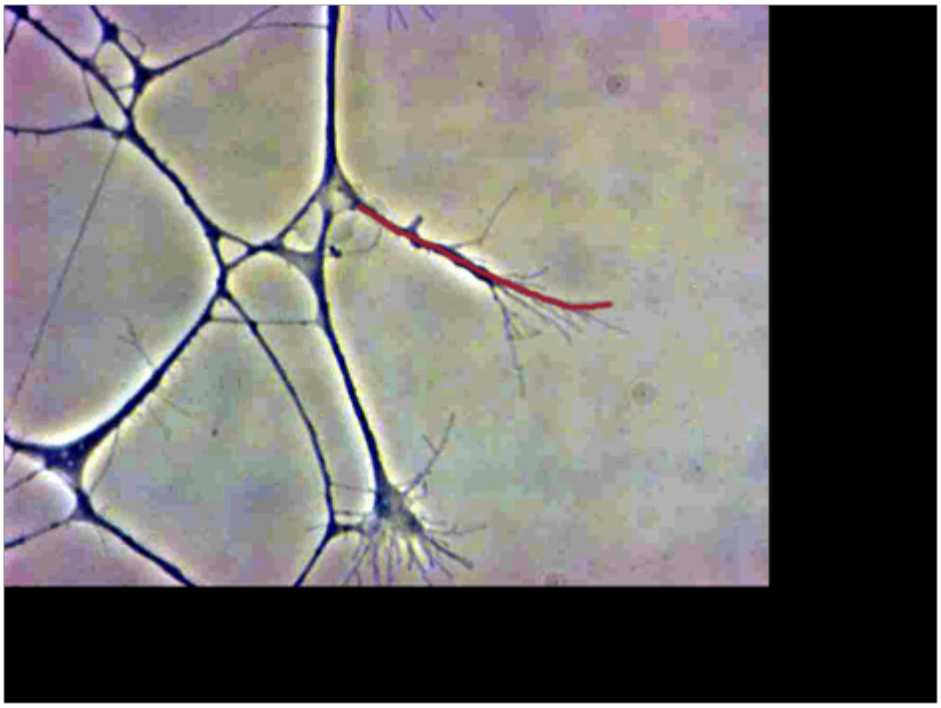

Figure 1B. Control neuron after 15minutes after 0.75 ml L-15 growth medium flow applied under total magnification of 40X

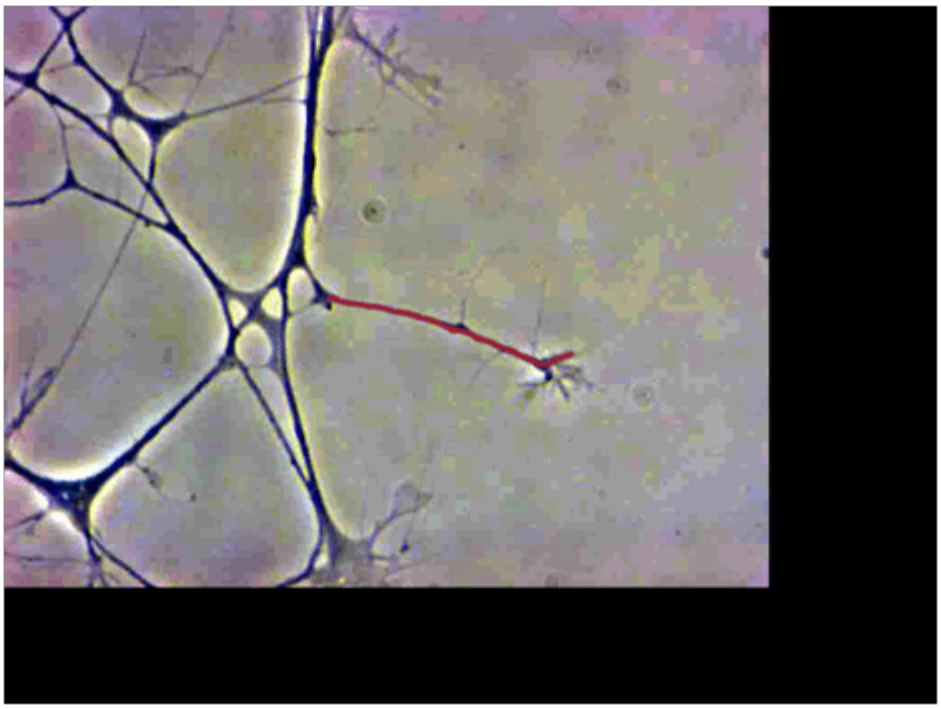

Figure 2AExperimental neuron at time 0 under total magnification of 40X

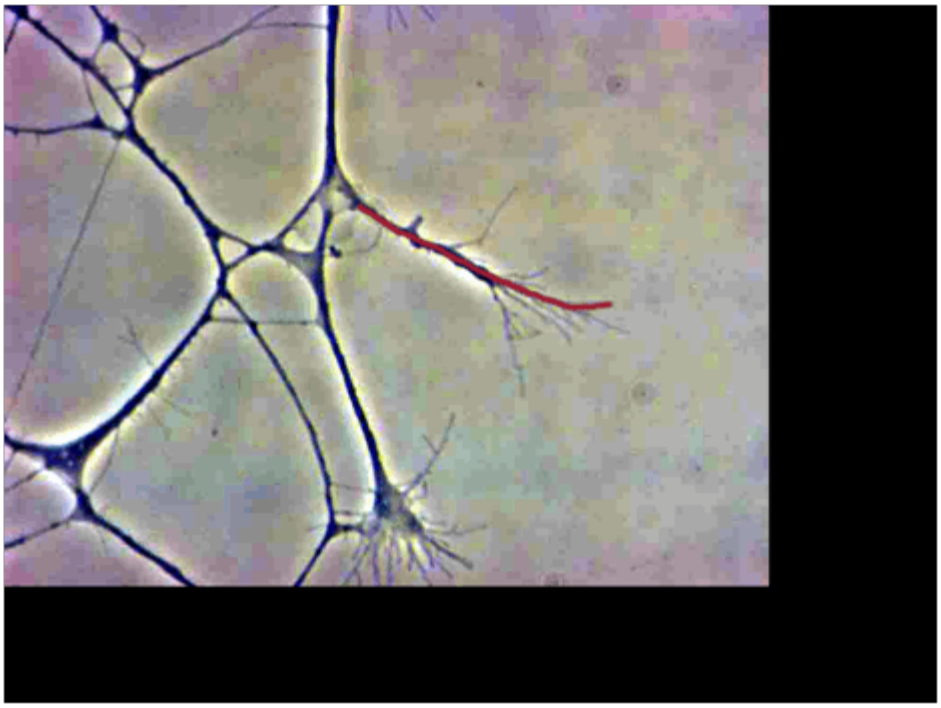

Figure 2B. Experimental neuron after15minutes after 0.75 ml of 100nM Mercuric Chloride growth and washed with 0.75 ml L-15 growth medium under total magnification of 40X

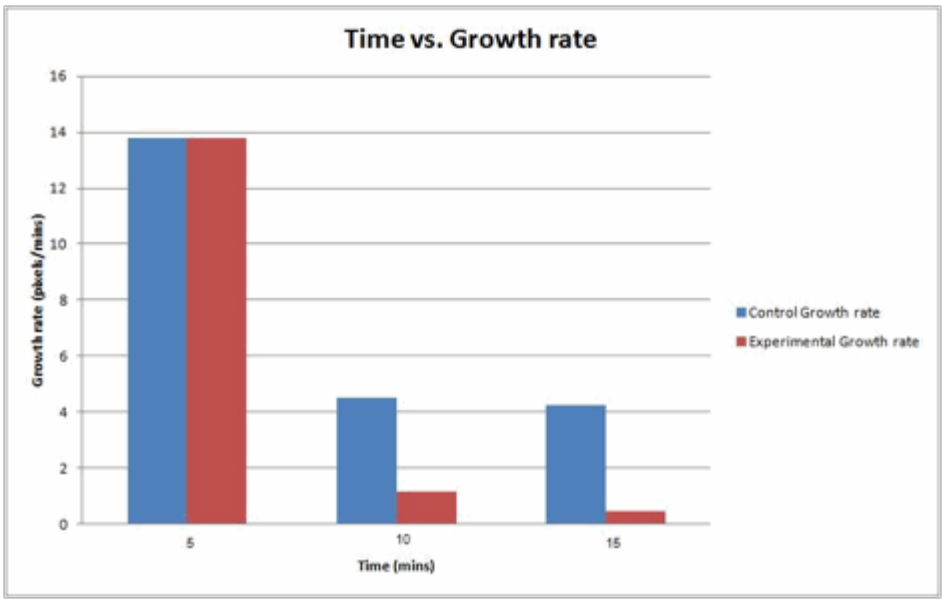

Figure 3. Growth rate of control and experimental neuron in 15 minutes

Discussions:

Data from this particular experiment supported the hypothesis. Figure 3 shows the growth rate of control and experimental neuron. Growth rate was obtained through distance of a neuron that was selected subtracted from the same neuron 5minutes later divided by the corresponding time. As shown in figure 3, it is suggested that the control neuron and experimental neuron experienced decrease of growth rate, however, unlike experimental neuron the control neuron did not have a dramatic decrease of growth rate after 5 minutes when mercury treatment was applied. This is

likely because experimental neuron was affected by the mercuric chloride toxicity. Although data supported the hypothesis, decrease of growth rate in control neuron was not expected since normal L-15 growth medium flow was applied instead of mercuric chloride. In this particular experiment control and experimental neuron both showed growth retraction in figure. Also, the data pool is not large enough to establish statistical significance, therefore, this experiment cannot conclude that mercuric chloride is the cause of growth retraction.

Although mechanisms of how mercuric chloride affects human nerve cells remain unknown, but there are studies that show mercuric chloride exerts toxic effects selectively on the brain (Guzzi et. al, 2008). In the future, the effect of mercuric chloride on the growth of the three filaments (intermediate filaments, actin filaments, and microtubules) of the cytoskeleton in nerve cell could be investigated. For example in a nerve cell, intermediate filaments play an essential role in cell growth. They are strong and ropelike. They strengthen cells against mechanical stress and the nuclear envelope is supported by a meshwork of intermediate filaments (Cooper, 2009). Since cytoskeletons like intermediate filament is vital to cell growth, an investigation with mercury treatment should be attempted along with more trials should be done to collect enough data to establish statistical significance to show that mercuric chloride causes decrease of growth rate and the mechanism in experimental neuron while control neuron with normal L-15 growth medium flow should shown increase of growth rate.

References:

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Kevin Hewitt has assisted me in obtaining data for this experiment in Bio 324.