Retraction in sympathetic neuronal growth cones when exposed to mercury

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Neurobiology Short Report

Bio324/Neurobiology

Wheaton College, Norton, Massachusetts, USA

November 30, 2011

Introduction

The nervous system along with all its complexities is an area in science that is constantly being investigated. However, an exciting and interesting feature that was focused on in this research report was the exploration and navigation of neuronal growth cones. The growth cone is located at the tips of developing neurties (Leong et al, 2011.) They are also the direct precursors to synaptic and neurosecretory terminals and undergo dramatic structural changes on target (Forscher et al, 1988).

The process of neurons that was studied was the retraction of growth cones.In collaboration with Jason Brown; retraction was defined as the shrinkage of the growth cone. In this study, the hypothesis that was tested was growth cones will retract after being exposed to mercury as measured by the area of the growth cone. This is significant as well as an interesting hypothesis to investigate because of research previously done by Leong et al. The research paper stated that in the presence of mercury the growth cones shapes will drastically change by either causing them to collapse upon themselves or not have a “distinct” growth cone shape (Leong et al, 2011).

Methylmercury pollutant primarily found in aquatic environments has also been shown to inhibit or disrupt various aspects of neuronal cell behavior and structural integrity. Dr. Benoit stated in her presentation, it has been seen in research that mercury impacts the developing fetus which drastically impacts the body at a cellular and biochemical level (Benoit, 2011). Another research study has shown that high concentrations of mercury will allow mercury to bind to microtubules which will therefore alter the normal dynamics of the growth cones (Bray and Chapman, 2011). The organisms that are being used to test this hypothesis are 10 day old sympathetic chick neurons, *Gallus gallus.* In the developing fetus this neurotoxin crosses the blood brain barrier targeting the developing brain. In this study, chick sympathetic neurons were treated with mercury for thirty minutes and analyzed to see whether retraction took place.

Materials and Methods

The materials that were used in the experiment to create the flow chamber were a one dish of cells, pipette bulbs, VALAP, a microscope slide, clean but not sterile short Pasteur pipettes, a dish of coverslip chips, a box of kimwipes, forceps, methyl mercury in HBSS, 1.5 mL Eppendorf tube clean but not sterile and a pair of safety glasses. The equipment that was used to collect data was a phase microscope (Nikon Eclipse E200 with phase optic imaged through Sony DFW-X700 with 1.0X C-mount), a heater, athermometer, the programBTV version 6.0b1 on iMac OS X version 10.5.8, Preview, and ImageJ 1.40.

The following methods were used to perform this experiment: Morris (2011a), Morris (2011b), Morris (2011 c), and Morris (2011 d). When imaging the cells at the imaging lab, three separate pictures were taken at two minute intervals when the heater was continuously blowing on the microscope slide. In addition, when imaging the cells, 0.7 mL of 100 nM mercury was added to the flow chamber. The mercury was left in the chamber for thirty minutes before 0.5 mL of buffer was exchanged into the flow chamber. In addition, three separate pictures were taken at two minute intervals while the heater was continuously blowing on the microscope slide at a temperature of 37°C but not greater than 42°C. In total six pictures were taken, three pictures before mercury exposure and three after mercury exposure. Therefore during analysis six pictures were analyzed for quantification. The methods for quantitation involved the area of the growth cone. Using the program ImageJ (2011d), the images taken during the experiment were adjusted for brightness and contrast to better allow detection of the growth cone to get the measurement of interest. To measure the area of the growth cone the image was opened with ImageJ. Straight, segmented, or freehand lines were the selected tool on the tool bar. Next the image was clicked and dragged to stretch out a line across the length of the growth cone. As stated before the growth cone was defined as the area located at the tips of developing neurites. Then analyze and measure were selected. The results window indicated the area of the growth cone in pixels. This number was recorded and these steps were repeated for additional pictures. Only one experimental trial was done. The data were collected by taking pictures at specific time points. The specific time points were decided based on the control experiment that was previously done prior to the experimental procedure. The data that was quantitated was the area of the growth cone and that was calculated by using the ImageJ program as mentioned above. The data were analyzed by looking at all the pictures that were taken during the experiment as well as the results of the area in each of those pictures. The experimental controls were the growth cones in Hanks solution to test whether retraction would occur in the growth cones. It was appropriate to use a control for this experiment to show that are solution and our growth cones actually work. If retraction was seen in the control experiment than that would indicate that the hypothesis could be tested.

Results

The data collected for this experiment show the effects of mercury before and after exposure of the growth cones. Before mercury was exposed to the growth cones, typical growth cone behavior was observed. These images show that the growth cone area was greater before the growth cones are exposed to mercury as compared to a thirty minute exposure to 100 nM mercury. These images represented growth cones on one slide. Figure 1 below shows the control before exposure to mercury.

Figure 1. Growth cone before mercury exposure. The growth cone is indicated by the black arrow. This figure clearly shows the growth cone which has been traced. This figure shows the “distinct” shape of a growth cone seen in neuronal.

Figure 1 significantly shows that exchanging the buffer did not cause the growth cone to retract.

Figure 2 depicts the effect mercury had on the growth cone after thirty minutes of exposure.

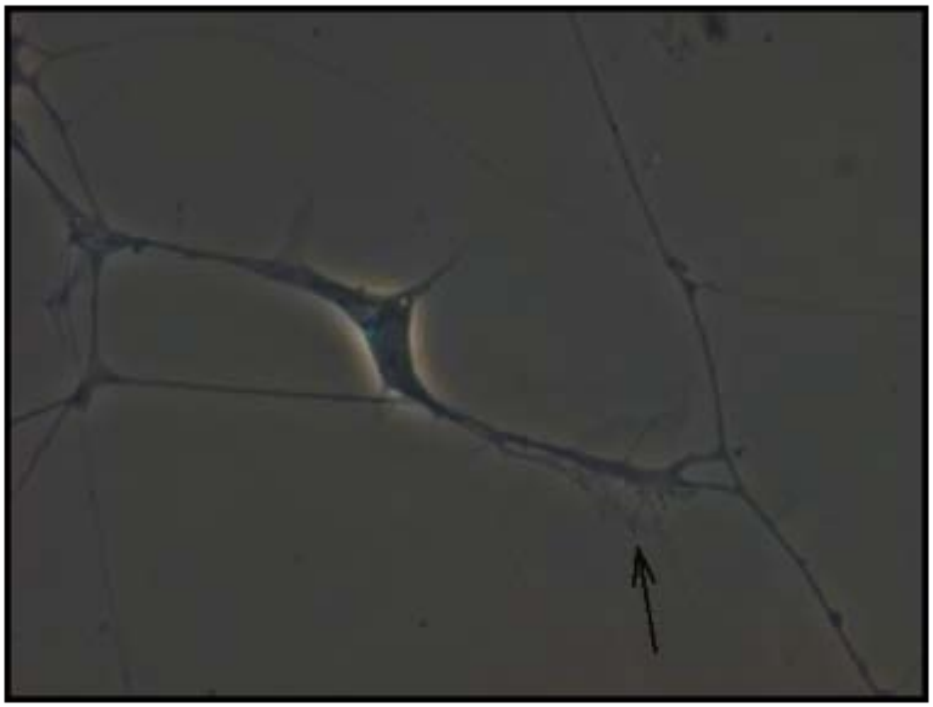

Figure 2. Growth cone exposure to mercury after 30 minutes. The growth cone is indicated by the black arrow. This figure shows how the axon has become elongated but does not show any retraction in the growth cone. Also notice that a new growth cone has formed than what was shown in the control.

As indicated in Figure 2, pre exposure to mercury results in an different growth cone than seen in Figure 1. Three images were taken to show the represented population of growth cones before exposure. Each image clearly indicated a normal growth cone. In addition, three images were taken to show the represented population of growth cone after they were exposed for thirty minutes. The growth cones looked different from the growth cones imaged pre exposure of mercury.

Figure 3 below shows a graphical representation of the growth cone area before and after a thirty minute exposure to mercury. Before exposure, the area of the growth cones was rather large. After exposure, the growth cones are decreased significantly.

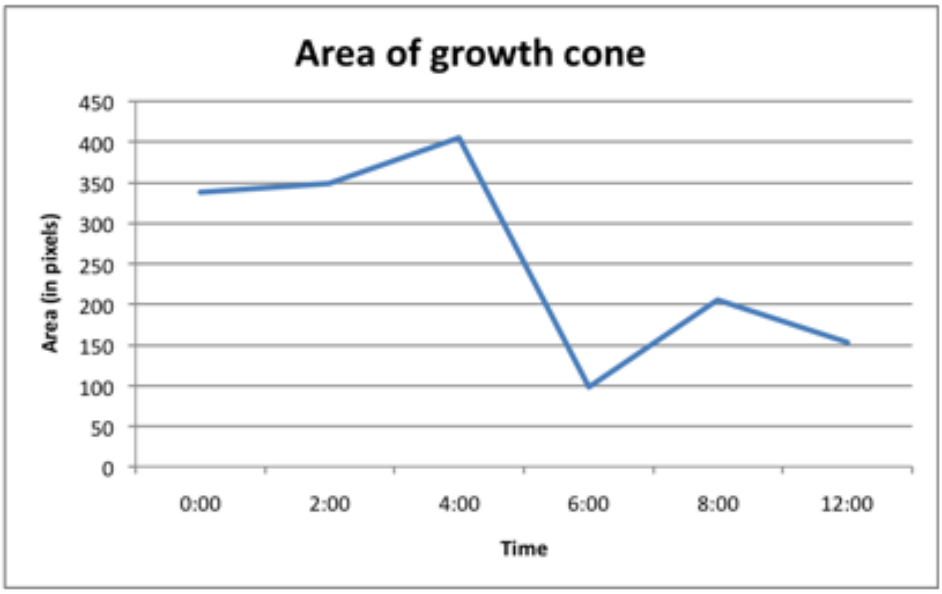

Figure 3. The area of growth cones before and after exposure to mercury. Mercury was added between time point 4 and 6. During the experiment 6 total pictures were taken in addition to a video. Notice that the area of the growth cone is greater at time points 1, 2, and 3 and then significantly drops at time point 4.

Table 1 shown below represents the pictures that were taken at specific time points. In addition, the addition of mercury is indicated as well.

Table 1. The N values for the points are indicated in this table. Six images were quantified for this experiment.

Points	Time
1	3:09 p.m.
2	3:11 p.m.
3	3:13 p.m.
Mercury added	3:17 p.m.
4	3:53 p.m.
5	3:55 p.m.
6	3:57 p.m.

It is important to note that the data represented above represent one experimental trail of growth cone exposure to mercury.

Discussion and Conclusion

This study resulted from two growth cones measured on one slide for thirty minutes. As a result, retraction was not seen in the growth cone when exposed to mercury for thirty minutes. Therefore, my hypothesis was refuted. Instead of seeing retraction of the growth cone, the growth cone grew in the presence of mercury. At the end of the thirty minutes, the axon elongated causing the same growth cone that was seen in Figure 1 to morph and form. This possibly implied that a new growth cone emerged but this was not the case as seen in Figure 2. In addition, the video that was taken during the thirty minutes did not indicate that the growth cone disappeared (Figure 1). The results show that retraction did not occur. The Graph 1 supports how the area of the growth cone changed but does not signify that retraction took place according to what was previously observed by Leong et al. However, the data suggests that the area of the growth cone decreased after being exposed to mercury for thirty minutes. If this experiment was repeated continuously and the results shown in this paper remained the same, the conclusion that possibly would be drawn is that the concentration of the mercury used in this experiment does not cause retraction in growth cones.

One possible error that could have affected the results of the experiment was the amount of mercury that actually entered the flow chamber. It is possible to say that the 0.7mL of mercury that was in the pipette wasn't in the flow chamber and could have possibly lingered around side of the slide that the mercury entered. Therefore, a significantly lower amount of mercury was exposed to the growth cone which resulted in no retraction of the growth cone to be seen. Due to this possible experimental error, it was crucial to have the pipette right up against the coverslip.

If this experiment was refined, a better apparatus to create a flow chamber may be beneficial. This would be essential so that the cells can be fully exposed to the mercury in order to get the results that were seen in the research. In addition, it may also be helpful to create a better apparatus to guarantee that the cells will be completely exposed to mercury but in this case they were not.

Future experiments should possibly investigate what proteins become present allowing the growth cone to grow in the presence of mercury. Also it would be interesting to see if mercury could possibly enhance or increase microtubule polymerization under these experimental circumstances.

References Cited

Acknowledgement of Jason Brown for collaboration of this experimental

Aletta, John M., and Lloyd A. Greene. “Growth Cone Configuration and Advance: A Time-Lapse Study Using Video-Enhanced Differential Interference Contrast Microscopy.” *The Journal of Neuroscience* 8.4 (1988): 1423-435. Print.

Bray, D., and K. Chapman. “Analysis of Microspike Movements on the Neuronal Growth Cone.” *The Journal of Neuroscienc*e 5.12 (1985): 3204-213. Print.

Forscher, Paul, and Stephen J. Smith. “Actions of Cytochalasins on the Organization of Actin Filaments and Microtubules in a Neuronal Growth Cone.” *The Journal of Cell Biology* 107 (1988): 1505-516. Print.

Leong, Christopher C. W., Naweed I. Syed, and Fritz L. Lorscheider. “Retrograde Degeneration of Neurite Membrane Structural Integrity of Nerve Growth Cones following in Vitro Exposure to Mercury.” *Neuroreport*12.4 (2001):733-37. Print.

Morris, R. L., and P.J. Hollenbeck. “Axonal Transport of Mitochondria along Microtubules and F-Actin in Living Vertebrate Neurons.” *The Journal of Cell Biology* 131.5 (1995):1315-326. Print.

Morris, Robert L., and Peter J. Hollenbeck. “The Regulation of Bidirectional Mitochondrial Transport is Coordinated with Axonal Outgrowth.” *The Journal of Cell Science* 104(1993): 917-27. Print.

Morris, R.L. (2011a) Neurobiology Bio324 Primary Culture of Chick Embryonic Peripheral Neurons 1: DISSECTION. Available athttp://icuc.wheatonma.edu/bio324/2011/LabProjProc/1.

Morris, R.L. (2011b) Neurobiology Bio 324 Primary Culture of Chick Embryonic Peripheral Neurons 2: OBSERVATION. Available at http://icuc.wheatonma.edu/bio324/2011/LabProjProc/2.

. Morris, R.L. (2011 c) Neurobiology Bio 324 Primary Culture of Chick Embryonic Peripheral Neurons 3: STAINING and OBSERVATION. Available at http://icuc.wheatonma.edu/bio324/2011/LabProjProc/3.

. Morris, R.L. (2011 d) Neurobiology Bio 324 Primary Culture of Chick Embryonic Peripheral Neurons 4: QUANTITATION.

. Notes from Professor Benoit’s presentation. (9/21/2011 in the Mars Center for Science and Technology, room 1114 at

Wheaton College).