The Effect of Methyl Mercury on Mitochondrial Activity in Axon

Guanjia Pan
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Introduction

There are two main classes of cells in the nervous system: nerve cells, or neurons, and glial cells, or glia (Kandel, 2013). A typical neuron has four defined elements: the cell body, dendrites, axon, and presynaptic terminals. The transmitting region of neurons is called axon. The axon typically extends some distance from the cell body and carries signals to other neurons. An axon can convey electrical signals over distances ranging from 0.1 mm to 2 m (Kandel, 2013). Mitochondria in axons are the main focus of this study. Mitochondria are described as "the powerhouse of the cell" because they generate most of the cell's supply of adenosine triphosphate (ATP), used as a source of chemical energy (Campbell, Neil A et al. 2006). Movement of mitochondria in axons can serve as a general model for how all organelles move: mitochondria are easy to identify, they move along both microtubule and actin tracks, they pause and change direction, and their transport is modulated in response to physiological signals (Hollenbeck and Saxton, 2006).

Mercury is a neurotoxin and that high levels of exposure can lead to serious illness and, in extreme cases, death (Environment Canada, 2010). The World Health Organization (WHO) has named mercury in the list of top 10 most dangerous chemicals. The inhalation of mercury vapour can produce harmful effects on the nervous, digestive and immune systems, lungs and kidneys, and may be fatal (WHO, 2013). Mercury also has effects on skin diseases and its concentrations in the river ecosystem do finally reach the humans through the food chain (The Pioneer, 2015).

Products like mercury thermometers, contaminated fish and shellfish are in people's daily life. The most serious effects of mercury in humans are damages in the nervous system (Costa, Fernanda do N et al. 2015). Since axons play a significant role of transmission of signals in the nervous system, it indicates that mercury also have effects on axons.

In this study, I tested the hypothesis that when exposed to methyl mercury, there will be less mitochondria activity in axons compared to axons that are not exposed to methyl mercury. The organism used for this particular study is *Gallus gallus* neuron culture. The reason using this organism is because of the similarity between the nervous system of this species to that of the human species. To test the mercury effect on *Gallus gallus* neuron is to estimate the effect on human species. The activity of mitochondria was studied in control axons with no exposure to methyl mercury and experimental axons that received exposure to methyl mercury. The mitochondria in the both control and experimental axons were observed and imaged by using Mitotracker, which is a florescent dye, and the dye is bright if there is an abundance of mitochondria in a particular area because of increased membrane potential (Chazotte, 2012). In this study, the dye is bright if mitochondria are more active in a particular region, and if mitochondria are less active, I expect to see a less brightness.

In previous studies (Leong et al, 2001) an educational film from the University of Calgary shows that brain neuron degeneration when they are exposed to mercury. And methyl mercury effects on mouse brain are well documented (Castoldi et al, 1996). My research is important because it will provide scientists another perspective to study the impact of mercury on neuron based on its effects on axon.

Materials and Methods

Materials:

Check materials from Neurobiology Bio324 Primary Culture of Chick Embryonic Peripheral Neurons 1: Dissection (Morris, 2015a) for the primary culture of neurons. In addition, used 4microMolar stock of Hg in HCl, diluted 1:100 to 40nM in HBSS for experimental group and dilution 1:100 of HCl in HBSS for control were also used in this study. Additional steps to grow more axons from the neurons were dissected 60 ganglia and 6 sympathetic chains, plated 12 dishes ganglia chains, and plated 36 dishes of mixed ganglia chunks and dissociated cells. Cells images were taken using an E200 Nikon Eclipse Microscope with phase optics and florescent light capabilities with a Sony DFW – x700 digital interface camera.

Methods:

Detailed steps of the procedure were carried out as Neurobiology Bio324 Primary Culture of Chick Embryonic Peripheral Neurons 2: OBSERVATION of LIVE UNLABLED CELLS. The data sets were collected in pairs. For control data, there was no methyl mercury exposure, where in the experimental data, the cells were exposed to a low dose of methyl mercury.

Detailed description of the steps taken to stain the cells were carried out as Neurobiology Bio324 Primary Culture of Chick Embryonic Peripheral Neurons 3, 2015: STAINING and OBSERVATION of LIVE CELLS. The specific steps are as followed: Extracted growth media, and set aside, then immediately replaced GM with about 1ml of MeHgCl for experimental group and 1ml of diluted HCl for control. Coverslips in petri dishes were then incubated for 15 mins at 37 degrees C, after 15 minutes, petri dishes removed from incubator. MeHgCl and HCl extracted from each dish. Then immediately replaced perturbations with HBSS to wash, and incubated petri dishes with HBSS for 5 mins. Repeated wash 2 more times for a total of 3 washes with HBSS with 5 minutes incubations. After 3rd wash, HBSS was replaced with GM, and then put petri dishes incubated for about 10 mins. Labeled

cells with Mitotracker, then removed GM from petri dish and set aside. Immediately replaced GM with about 1 ml of Mitotracker. Then, incubated for 15 mins at 37 degrees C. After 15 mins, extracted Mitotracker and almost immediately replaced with HBSS to wash. Then incubated for 5 mins at 37 degrees C. Repeated wash 2 more times for a total of 3 washes with HBSS, with 5 minutes of incubation at 37 degrees C in between each wash. After 3rd wash/incubation for 5 mins, HBSS was extracted and almost immediately replaced with GM. Then incubated for about 10 mins at 37 degrees C. Then created chip chamber sealed with VALAP.

Software SPOT was used to image axons at 40x objective lens in Phase 2. Then ImageJ was used to measure the brightness of stained mitochondria in axons from the images. Detailed Steps are as follow: The best and most focused images were opened in the ImageJ (one control, one experiment), the magnifying glass from the tool bar was used to magnify the image, then “point or multiple point” from the tool bar was used to locate each mitochondria on the axons in a particular area. Click analyze, then click measure to record the brightness of each individual mitochondria. Overall 20 of the brightness of stained mitochondria were measured and recorded (10 for the control group, and 10 for the experimental group), then the mean value of these measurements was calculated and a bar graph was generated by using Excel.

Results

The data represent the activity of 20 different mitochondria in axon. Ten individual data sets were observed from the control with no methyl mercury exposure and ten individual data sets were collected from the experimental axons with methyl mercury exposure.

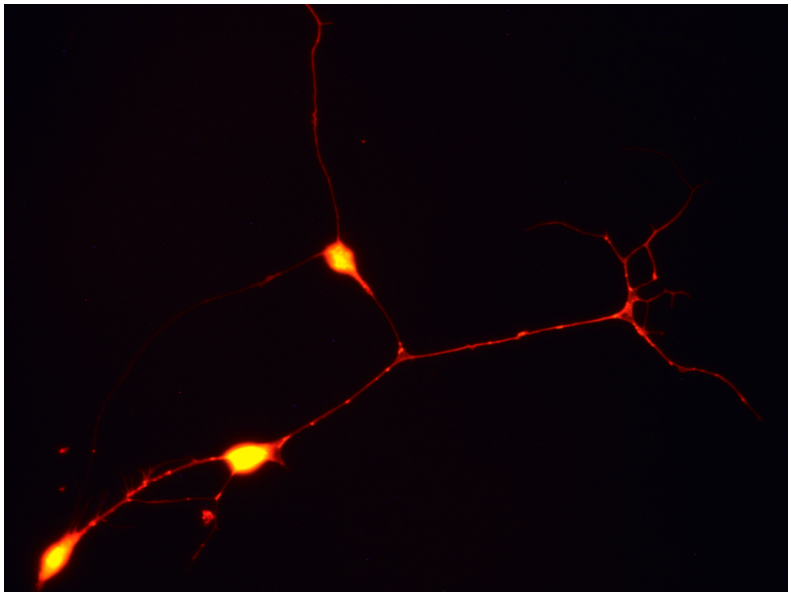

Figure 1: This figure shows the fluorescence of the control group, which means the axons in this image were not exposed to methyl mercury. The Mitotracker stained mitochondria with bright and orange color.

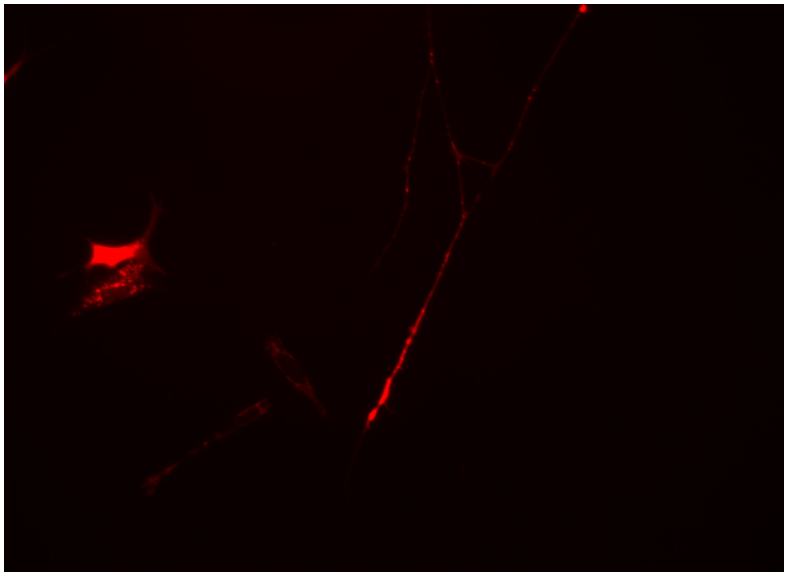

Figure 2: This figure shows the fluorescence of the experimental group, which means the axons in this image have received methyl mercury exposure. The Mitotracker stained mitochondria with bright and orange color.

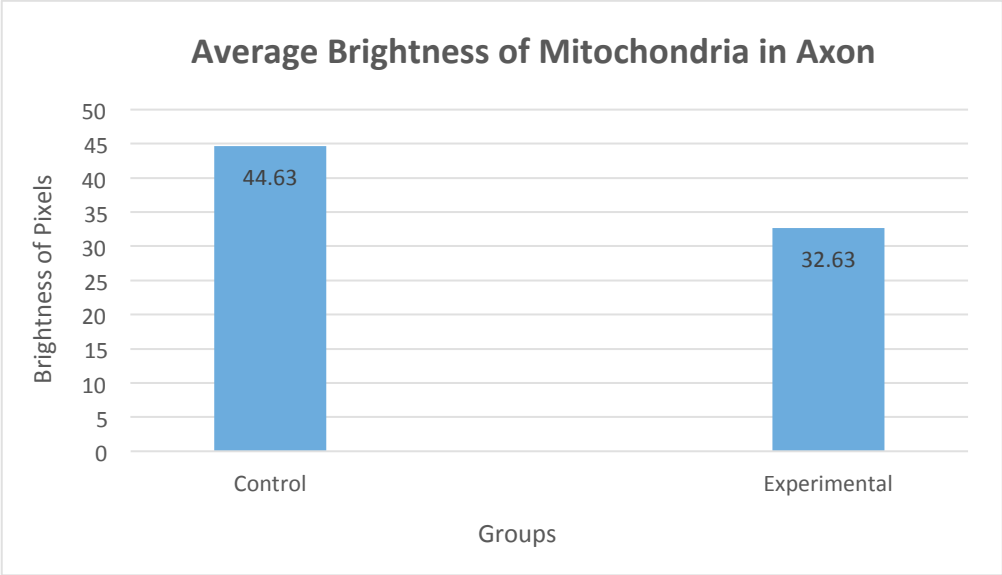

Figure 3: This figure compares the mean brightness between the control and experimental group when stained with Mitotracker. The experimental group (n = 10) has lower average brightness than the control group (n = 10).

Discussion

The result of this study support my hypothesis, because when the axons were exposed to methyl mercury, the brightness of mitochondria decreased as shown on Figure 3. Those differences in brightness correspond to the change mitochondrial activity in axons (Chazotte, 2011), and decreased mitochondrial activity suggests less ATP production. As mentioned above in the introduction, axons are important for transmitting signals to different neurons. If axons are exposed to methyl mercury, reducing mitochondrial activity in axons, there will be probably not enough ATP for axons to function, then it is reasonable to expect these disorders and malfunction in the nervous system.

If the experiment was repeated a thousand times and the same results were collected from the data set that all experimental data are statistically significant different than the control data, there will be a much stronger evidence to suggest and support the hypothesis, and I will be more convinced that the result are more accurate.

If I had that a great amount of data enough that I were convinced that all the trends and differences between experimentals and controls were real, on the cellular standpoint I would use the data to support the hypothesis, and it would be better to also analyze the data and explain it in molecular level.

There may be some source of error in this experiment. First, when coverslips in petri dishes were incubated, the temperature of incubation was around 34 degree C instead of 37 degree C, this may serve as additional variable for this study. Second, little amount of methyl mercury may leftover after the three washes, and thirdly when observing the image of control groups, the experimental coverslips was not stored properly, and the experimental was not being observed until 30 minute after, this may affect the activity of mitochondria on axons. In this study, the difference in brightness on average between control and experimental should not be affected, because the source of error may affect both groups. To refine this experiment, the washes of methyl mercury should increase, and the control coverslip and experimental coverslip should be observed and measured at the same time.

Future experiments should focus on different amount of methyl mercury effect on axon growth, it should be interesting to find out the minimum amount of mercury that can affect neuron. Additionally, the communication between neuron and axon when exposed to methyl mercury should be a great topic, and compare results to Wheaton alumni on the effect of mercury on neuron-glia interaction (Fess, 2006).

References Cited

Campbell, Neil A.; Brad Williamson; Robin J. Heyden. (2006). Biology: Exploring Life ISBN 0-13-250882-6.

Castoldi AF, Candura SM, Costa P, Manzo L. Costa LG. Interaction of mercury compounds with muscarinic receptor subtypes in the rat brain. Neurotoxicology. 1996, 17(3-4):735-741

Chazotte, B. (2011). Labeling mitochondria with MitoTracker dyes. Cold Spring Harbor Protocols, 2011(8), pdbprot5648

E. R Kandel, J. H. Schwartz, and T.M Jessell, S. Siegelbaum, A.J Hudspeth. Principles of Neural Science. 5th Edition. McGraw Hill. 2012.

Environment Canada, (2010). Retrieved from: http://www.ec.gc.ca/mercuremercury/default.asp?lang=En&n=9A4397AD-1

Fernanda do N. Costa,Maria Graças A. Korn,Geysa B. Brito,Stacy Ferlin,Anne H. Fostier. (2015) Preliminary results of mercury levels in raw and cooked seafood and their public health impact. Food Chemistry. 2015

Fess, Juliana. 2006. The Effect of Mercury on Neuron-Glia Interaction. Retrieved from: http://icuc.wheatonma.edu/bio324/2006/jfess/index.html.

Hollenbeck, Peter J. and Saxton, William M. (2006). The axonal transport of mitochondria. J Cell Sci, 5411–5419.

Morris, R.L. (2015a) Neurobiology Bio324 primary culture of chick embryonic peripheral neurons 1: dissection. Retrieved from: http://icuc.wheatoncollege.edu/bio324/2015/NBlabPrimaryTissCultureProc1Dissn2015

Morris, R.L. (2015b) Neurobiology Bio324 primary culture of chick embryonic peripheral neurons 2: observation of live unlabled cells. Retrieved from: http://icuc.wheatoncollege.edu/bio324/2015/NBlabPrimaryTissCultureProc2ObservnOfLliveUnlabeledCells2015.htm

Morris, R.L. (2015c) Neurobiology Bio324 primary culture of chick embryonic peripheral neurons 3, 2015: staining and observation of live cells. Retrieved from: http://icuc.wheatoncollege.edu/bio324/2015/NBlabPrimaryTissCultureProc3StainAndObserv2015.htm.

The Pioneer. “Biggest Odisha festival is a painful hurting time” New Delhi (2015). Print.

WHO (World Health Organization). (2013) Fact Sheet