# The effects of methyl mercury on mitochondrial voltage in embryonic glial cells

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## Introduction:

Mitochondria are located in high density within regions of the neurons that need increased ATP for function (Verburg, 2008). They are abundantly found and are distributed by regulated transport in neurons. Mitochondria take on various important roles in the cell. Some of the most important roles include the production of ATP by oxidizing products like glucose, pyruvate, and NADH, which is also known as cellular respiration. Mitochondria also play a central role in cellular energy metabolism (Krauss, 2001). One of the greatest interests of mitochondria is their ability to regulate cell life and death transitions. Mitochondrial membrane potential is crucial to the cell life-death transition. Changes in this membrane potential can indicate the onset of cell death (Griffiths, 2000). Another study has also gathered results that show methyl mercury having a profound reduction in mitochondrial membrane potential and an overall decrease in the dimension of mitochondria (Shenker, 1999).

Methyl mercury (MeHg) is known to be extremely harmful to living organisms. It is commonly found as a form of mercury in the environment. Methyl mercury has shown to directly affect the release of neurotransmitters from presynaptic nerve terminals. These changes may be controlled by changes in the intracellular concentration of calcium ($Ca_2$) (Atchinson, 1994). An exposure to methyl mercury can disturb the regulation of calcium from an intracellular pool, increase the permeability of the plasma membrane to calcium, and block the plasma membrane voltage-dependent sodium and calcium channels. Mitochondria are known to be the "powerhouses" of cells and to produce ATP for the cells to live. MeHg can hinder that production as it inhibits many mitochondrial enzymes while it depolarizes the membrane of the mitochondria. This allows the ATP production to be decreased and the buffering capacity of calcium to also reduce (Atchinson, 1994).

Both in vivo and in vitro studies have developed results that indicate mitochondria as a site of MeHg neurotoxicity. Many studies have been performed on rats to see the MeHg effect on the mitochondria. With one in vivo study, mitochondrial function was impaired during the symptomatic phase with the treatment of MeHg. Synaptosomes from the rats that were treated with MeHg in vitro were seen to have reduced rates of respiration (Atchinson, 1994). Another study using rat striatal synaptosomes shows that using the application of methyl mercury reduced mitochondrial metabolic

function and mitochondrial membrane potential. Reduction of mitochondria metabolic function was seen greatly after the exposure to MeHg. The exposure to MeHg also reduced the membrane potential in synaptosomes of the rats (Dreiem, 2005). From these studies, we can hypothesize that if the embryonic neurons are exposed to MeHg, then there will be a reduction in voltage and membrane potential seen in the mitochondria located in the glial cells. Glial cells are slightly different than nerve cells. Glia cells are usually a bit smaller but more abundant than nerve cells. Their functions are to define synaptic contacts and maintain the neuron's signaling abilities (Purves, 2001). We expect to see a difference of brightness between the cells that are not exposed to MeHg, and the cells that are treated with MeHg.

# Materials and Methods:

For the dissection of embryonic cells, we referred to "Primary Culture Of Chick Embryonic Peripheral Neurons 1: DISSECTION (Morris, 2014a). To view our live dissected ganglia cells and to locate glial cells, we followed "Primary Culture of Chick Embryonic Peripheral Neurons 2: OBSERVATION of LIVE UNLABELED CELLS" (Morris, 2014b). To prepare the cells for our procedure for this experiment, we took our control and experimental plates and removed the DMEM (growth medium). Then, we added more DMEM to the control and added DMEM plus the MeHg to our experimental plates. We then incubated our dishes for ten minutes. We removed them from the incubator and extracted the liquid in both and added Mitotracker stain to each dish. We then incubated those for another ten minutes, removed the Mitotracker, and added more growth factor. We made some changes to our methods between experimental days to ensure better cell growth. We changed the poly K treatment timing of the cover slide to three or more hours so it would be more "sticky". We also increased the Laminin treatment of the cover slides for two hours. The amount of glutamine, which allows more breakdown, was increased from 2mM to 4mM. Along with this, we made sure the shelf was specially isolated so the cells did not move around in the incubator. More ganglia and sympathetic chains were plated to give us better density and more of a chance to grow cells. We gave the cells 38 hours instead of 24 hours, in order for cells to attach to the petri dishes.

To gather our information about mitochondrial charge distribution in glial cells, we used fluorescent microscopy. We studied these cells with a high dose of MeHg (40nM MeHg in DMEM). With the fluorescent microscopy method, we stained the mitochondria and observed mitochondrial membrane charge in living neurons. Our staining and microscopy methods followed "Primary Culture of Chick Embryonic Peripheral Neurons 3: STAINING and OBSERVATION of LIVE CELLS" (Morris, 2014c). We used a Sony DFW (1.0x c-mount) camera processed by Spot TV software to capture our images. We then viewed our cells with mercury and fluorescence. We compared those to cells that were not treated with mercury. We observed our pictures and used 'Image J' to create histograms. We produced these to get the average

brightness for each image and normalize the background colors. For each image captured, we used the same exposure time, which was 525.5 ms. To normalize the background we used 'Image J' and opened both of the images we were comparing. We then selected a region of the picture and measured the same width and height of a box in both images. Then, we clicked "analyze" and created a histogram for these boxed sections of the images. This was created so we could get the mean, or average brightness. We then divided the average brightness of one picture by the average brightness of the other picture. With this, it gave us the ration of how much brighter one of the images was over the other. We then multiplied that to make both images equal in background. From here, we were able to analyze each image and determine mitochondrial brightness.

# Results:

For our results, we were able to determine the average brightness of the mitochondria located in the glial cells. By examining the brightness, we could determine if methyl mercury had an effect on the charge of the mitochondria in the cells. The brightness was measured in fluorescent cells that were treated with methyl mercury and then were compared to untreated, fluorescent neurons (control).

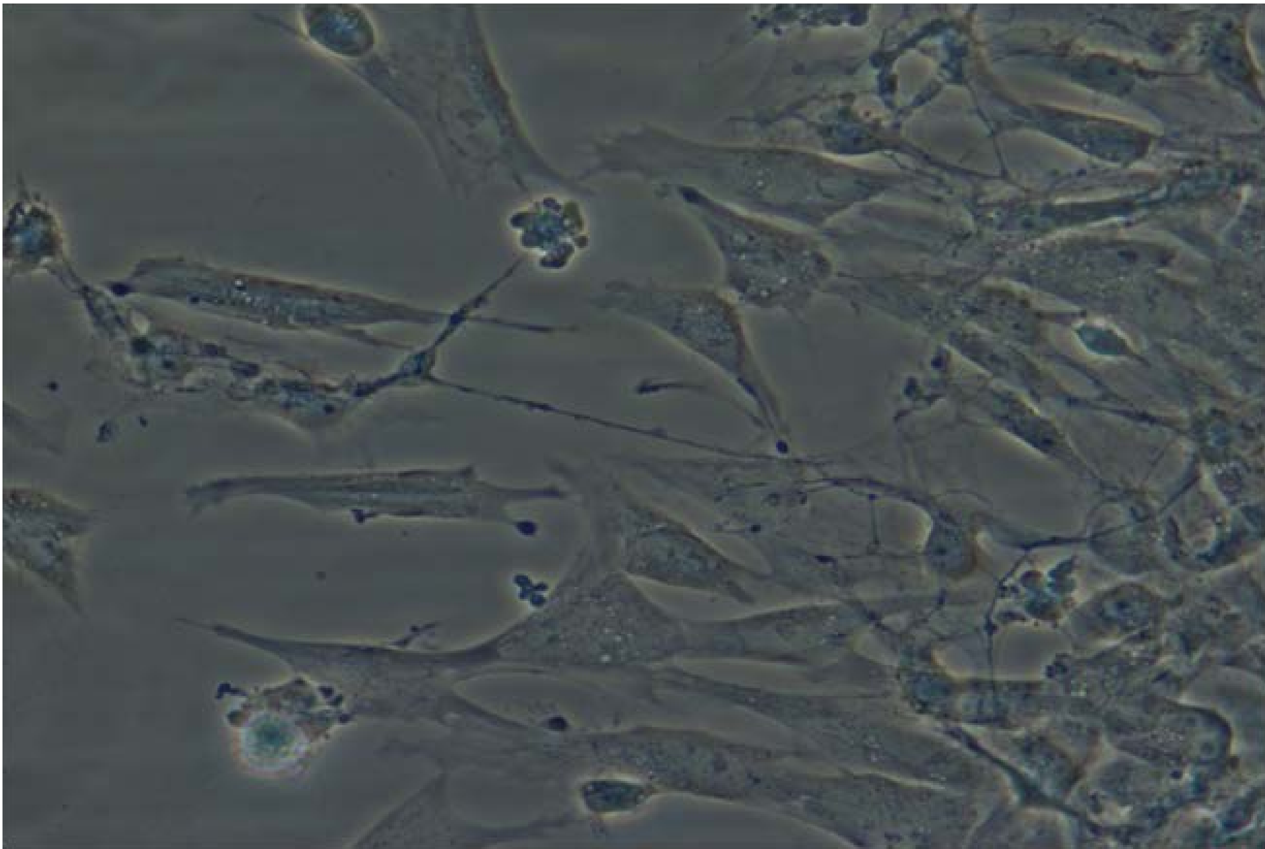

**Figure 1**: Neuron and glial cells under transmitted light. This image was captured using a Sony DFW x 700 with a 1.0 c-mount camera, also processed with BTV Software. Image was gathered in collaboration with Moya Willis.

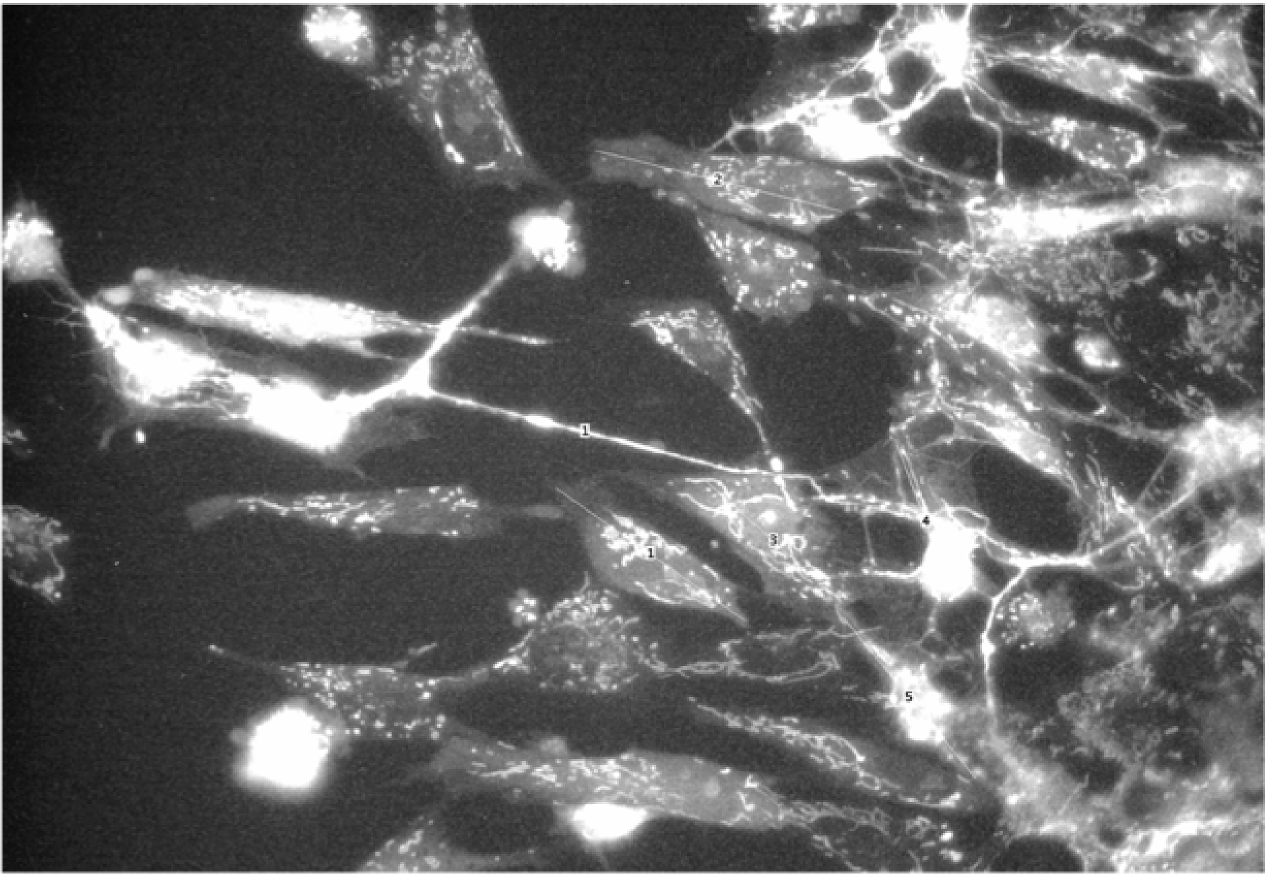


**Figure 2**: Neuron and glial cells (Figure 1) under fluorescent scope using a Sony DFW x 700 with a 1.0 c-mount camera, also processed with BTV Software. This image was processed with an exposure time of 525.2 ms. This sample includes five fluorescent glial cells (not treated with MeHg), which are measured and labeled with a number. Image was gathered in collaboration with Moya Willis.

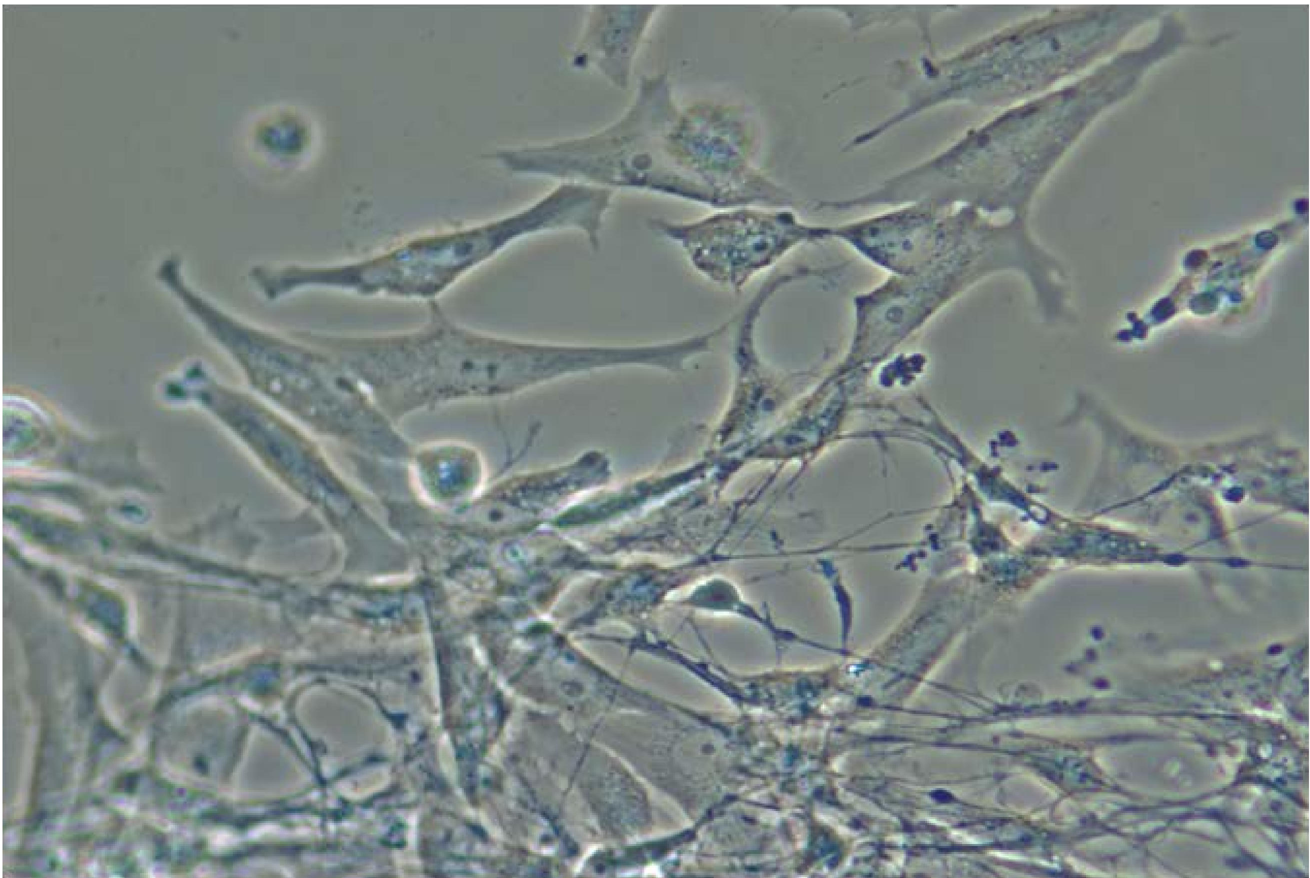

**Figure 3**: Neuron and glial cells under transmitted light, treated with a high dose of MeHg (40nM). This image was captured using a Sony DFW x 700 with a 1.0 c-mount camera, also processed with BTV Software. Image was gathered in collaboration with Moya Willis.

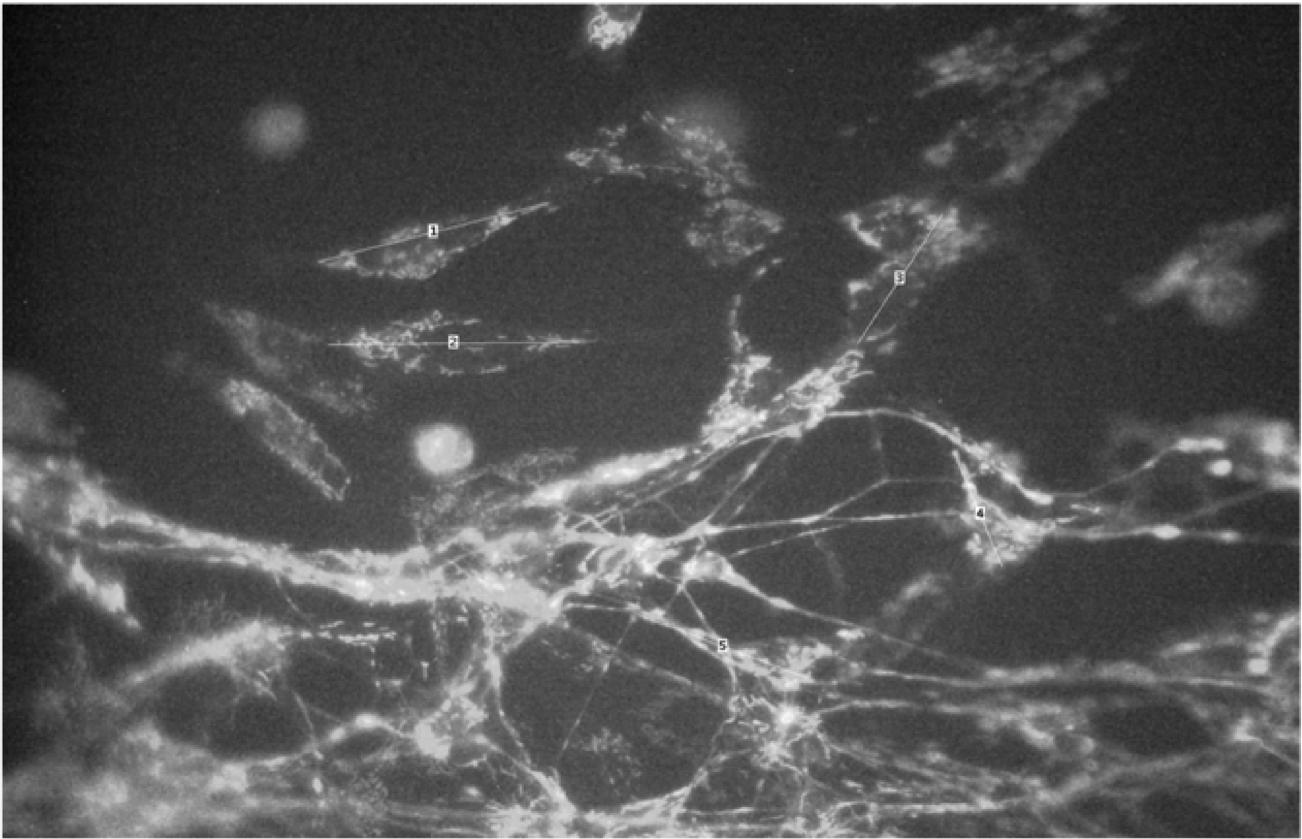


**Figure 4:** Neuron and glial cells (Figure 3) under fluorescent scope using a Sony DFW x 700 with a 1.0 c-mount camera, also processed with BTV Software. This image was processed with an exposure time of 525.2 ms. This sample includes five fluorescent glial cells that are treated with a high dose of MeHg (40nM), which are measured and labeled with a number. Image was gathered in collaboration with Moya Willis.

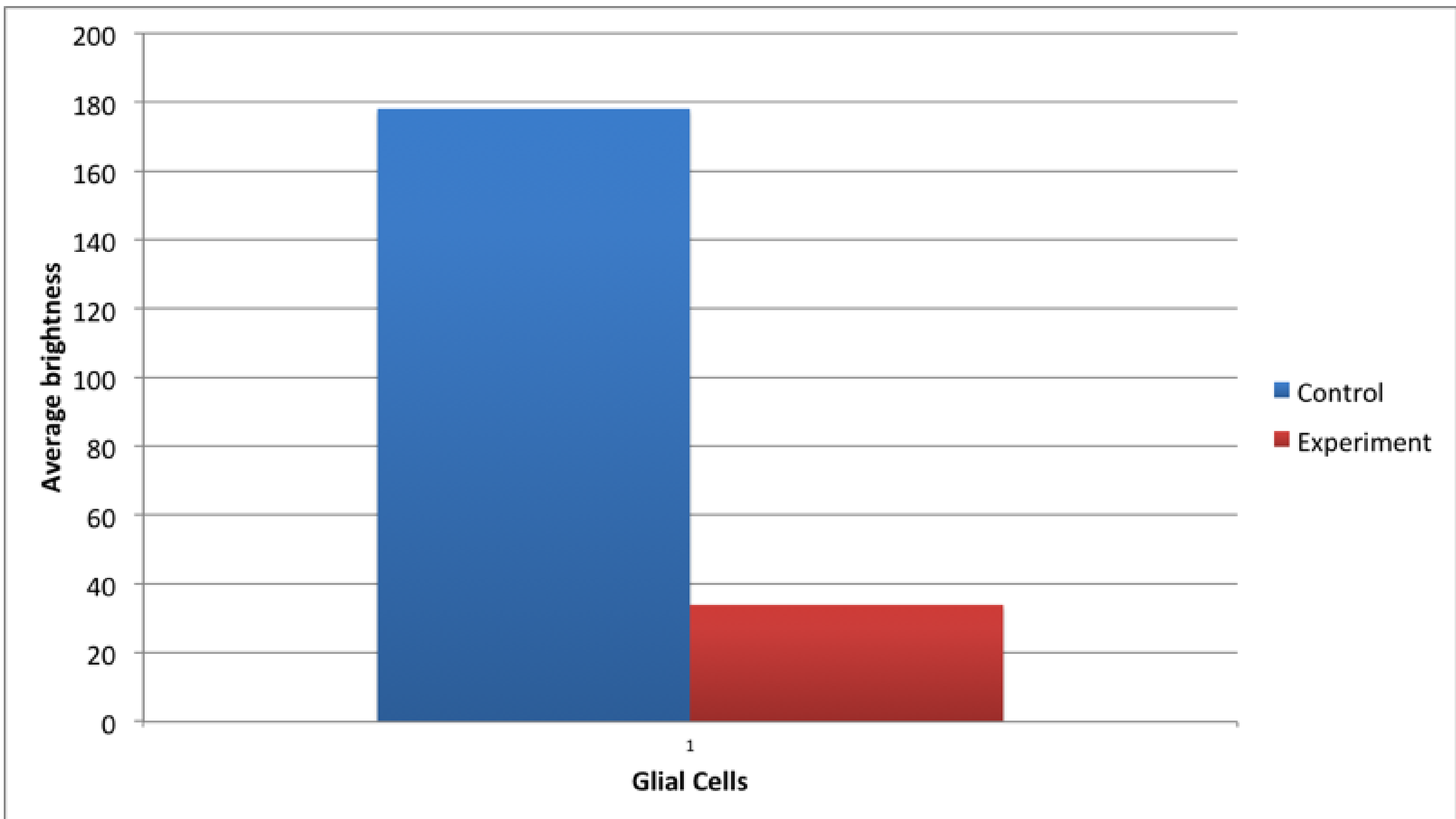


**Figure 5:** The average of brightness from the control and experimental images.

# Discussion:

By analyzing our images, we can see that the difference in brightness from the control to the experimental is very apparent. Because the background of each image is normalized, the brightness of the glial cells is accurately displayed. In figure 2, we see the glial cells to be very bright and illuminated. In figure 4, the glial cells are much more dull and do not have as bright of a glow. Figure 5 shows a bar graph that depicts the difference in average brightness between the two samples. The average brightness of the control is much higher. We assume that the difference in brightness is due to the decrease of charge caused by the methyl mercury.

The data that has been gathered can support the hypothesis stating that glial cell mitochondrial brightness will decrease when exposed to methyl mercury. The density of mitochondria relates to the light brightness emitted from Mitotracker with fluorescent lighting. A study has shown that MeHg favorably accumulates in glial cells, especially astrocytes (Aschner, 2000). By analyzing our data, and with this study, we can suggest that the mercury has settled heavily in these glial cells. With the idea that the MeHg has accumulated in the glial cells, we can also determine the reason for the dimness shown in figure 4. This figure can also indicate that the methyl mercury exposure has a significant impact at a cellular level. However, with our results it is quite unclear of the impact that the chemical has on an organism.

By producing images with fluorescent microscopy, we were able to notice differences in mitochondrial brightness

caused by methyl mercury exposure. Although neurons have traditionally been responsible for the release of ATP in the central nervous system, glial cells have recently been shown to release ATP. This study presents the possibility that glial cells may be able to modulate neuronal activity (Newman, 2003). Our results can also suggest that the methyl mercury exposure may have caused the decline of ATP production by glial cells, resulting in the decrease of the negative charge and membrane potential found in mitochondria. This decrease in negative charge is responsible for the appeared dimness of the glial cells. Possible future experiments may study specific types of glial cells that are exposed to methyl mercury. Other studies may also look at a variety of concentrations of methyl mercury needed in order to impact glial cells and the overall organism.

## References


Close collaboration with Moya Willis.

Aschner, M. "Methyl mercury Alters Glutamate Transport in Astrocytes." *Neurochemistry International* 37.2-3 (2000): 199-206.

Atchinson, W. D., Hare, M.F. "Mechanisms of Methylmercury-induced Neurotoxicity." *PMC* 8.9 (1994): 622-29.

Dreiem, A. "The Effects of Methylmercury on Mitochondrial Function and Reactive Oxygen Species Formation in Rat Striatal Synaptosomes Are Age-Dependent." *Toxicological Sciences* 87.1 (2005): 156-62.

Griffiths, E. "Mitochondria — Potential Role in Cell Life and Death." *Cardiovascular Research* 46.1 (2000): 24-27.

Krauss, Stefan. "Mitochondria: Structure and Role in Respiration." (2001): 1-6. Web. <http://www.med.ufro.cl/clases_apuntes/cs_preclinicas/mg-fisica-medica/sub-modulo-1/Mitochondria.pdf>.

Morris, R. "Neurobiology Bio324." *Primary Culture Of Chick Embryonic Peripheral Neurons 1: DISSECTION*. N.p., n.d. Web. 22 Apr. 2014a. http://icuc.wheatoncollege.edu/bio324/2014/morris_robert/BIO324_Lab_Proc_1_Dissection_2014.htm

Morris, R. "Neurobiology Bio324." *Primary Culture Of Chick Embryonic Peripheral Neurons 2: OBSERVATION of LIVE UNLABLED CELLS*. N.p., n.d. Web. 22 Apr. 2014b. http://icuc.wheatoncollege.edu/bio324/2014/morris_robert/BIO324_Lab_Proc_2_ObserveLiveCells_2014.htm

Morris, R. "Neurobiology Bio324." *Primary Culture Of Chick Embryonic Peripheral Neurons 3: STAINING and OBSERVATION of LIVE CELLS*. N.p., n.d. Web. 22 Apr. 2014c. http://icuc.wheatoncollege.edu/bio324/2014/morris_robert/BIO324_Lab_Proc_3_StainAndObserv_2014.htm

Newman, Eric A. "Glial Cell Inhibition of Neurons by Release of ATP." *The Journal of Neuroscience* 5.23 (2003): 1659-666.

Purves D, Augustine GJ, Fitzpatrick D, et al., editors. Neuroscience. 2nd edition. Sunderland (MA): Sinauer Associates; 2001. Neuroglial Cells. Available from: http://www.ncbi.nlm.nih.gov/books/NBK10869/

Shenker, B. "Induction of Apoptosis in Human T-Cells by Methyl Mercury: Temporal Relationship between Mitochondrial Dysfunction and Loss of Reductive Reserve." *Toxicology and Applied Pharmacology* 157.1 (1999): 23-35.

Verburg, J., and P. J. Hollenbeck. "Mitochondrial Membrane Potential in Axons Increases with Local Nerve Growth Factor or Semaphorin Signaling." *Journal of Neuroscience* 28.33 (2008): 8306-315.

I have abided by the Wheaton Honor Code in this work.

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