

# Effects of Methyl Mercury on Mitochondrial Distribution in *Gallus gallus* Glia

Lauren McClelland

Neurobiology Short Report

Bio324/Neurobiology

Wheaton College, Norton, Massachusetts, USA

April 23, 2014

## **Introduction:**

Glial cells are a type of neuronal cell found in the central nervous system (Michaud, 2013). They are the most abundant cell in the nervous system. Glia regulate the concentrations of ions and neurotransmitters around neurons, support signaling, act as phagocytes and provide a framework of support for neurons (Martini & Bartholomew, 2007). The two most abundant glial cells in the central nervous system are oligodendrocytes and astrocytes. Oligodendrocytes myelinate neurons, whereas astrocytes regulate the environment and modulate synaptic signaling (Kandel, Schwartz, Jessell, Siegelbaum & Hudspeth, 2000).

Mitochondria, powerhouses of cells, are cytoplasmic organelles. They produce 95% of the ATP required for the cell (Martini & Bartholomew, 2007). These organelles move in almost all eukaryotic cells, including in glial cells. In neuronal cells, mitochondria are of particular importance because problems can arise during the long range transport of organelles and neuronal cells have specialized energetic demands. The long processes of glia are susceptible to improper distribution of mitochondria because both the pre- and post-synapse have high demands for calcium buffering and energy (Lovas & Wang, 2012). It has been found that exposure to methyl mercury causes increased mitochondrial membrane permeability. The increase in permeability leads to an elimination of the mitochondrial inner membrane potential, causing severe damage and decreased ability of the mitochondria to function normally (Yin et al., 2007).

In this study we tested the hypothesis that in the presence of increasing amounts of methyl mercury, the mitochondria of the glial cells will move farther from the nucleus. The resting levels of calcium in the cell allow mitochondrial movement. When the concentration of cytosolic calcium increases, the mitochondria are inhibited from moving in both neurons and non-neuronal cells. This is because in areas of high calcium concentration there are lower ATP concentrations. The mitochondria stop moving in these areas to increase the amount of ATP and buffer the calcium concentration (Lovas & Wang, 2012). Methyl mercury noncompetitively and irreversibly suppresses calcium

entry (Atchison & Hare, 1994). Since methyl mercury blocks calcium from entering the cell, the intracellular calcium concentration would not increase. The inhibition of calcium entering the cell would keep the cell from periodically increasing its calcium concentration. This would cause the mitochondria to move more than in their normal environment, where they might sporadically be prevented from movement due to the increased concentrations of calcium.

Methyl mercury is a neurotoxicant (Leong, Syed & Lorscheider, 2000). The nervous system is the most sensitive target organ for the compound. Methyl mercury exposure is associated with many adverse health effects in animals and humans, especially in developing organisms. It impairs neurological development in fetuses and young children by impacting cognitive thinking, fine motor skills and memory. Methyl mercury is able to pass through the placenta, so a mother's consumption of fish that may have the compound can result in harming the developing brain and nervous system of the child. Detrimental health effects from exposure to methyl mercury include impairment of peripheral vision and speech, lack of coordination and muscle weakness ("Methylmercury," 2012). Since methyl mercury targets the nervous system in which glia are abundant, studying the effects of methyl mercury on glial cells is an important step in finding a solution to mercury poisoning.

In this study we treated embryonic chick peripheral glia with different levels of methyl mercury solutions and observed the change in the distribution of mitochondria by measuring their distances from the nuclei. The organism used in this study was *Gallus gallus*.

## **Materials and Methods:**

### *Cell Slide Preparation and Creation*

*Gallus gallus* embryonic sympathetic nerve chains and dorsal root ganglia were used. Culture of peripheral neurons and glia and coverslip preparation was performed with protocol by Morris' "Primary Culture of Chick Embryonic Peripheral Neurons 1: Dissection" (Hollenbeck & Morris, 2014). However, changes were made using DMEM in place of HBSS, poly-K treatment of coverslip for 1-3 hours, laminin treatment of coverslip for 1-2 hours, 50-200 ng/ml NGF, 2-4 mM glutamine, and 40-60 ganglia and 4-6 sympathetic chains incubated for 24-38 hours. All incubations occurred at 37 degrees Celsius.

### *Observation Chamber*

An observation chamber was created using protocol from Morris' "Primary Culture of Chick Embryonic Peripheral Neurons 2: OBSERVATION of LIVE UNLABELED CELLS" (Morris, 2014).

### *Control*

The petri dish containing the cover slip in growth medium was taken from the incubator and growth medium was removed. 500 ml DMEM and 10 ml 236 uM Mitotracker was added to the petri dish, which was then incubated for 10 minutes. At that time the DMEM and Mitotracker solution was removed, and the coverslip was washed three times with DMEM. Each of the three washes was one minute long. DMEM was then added back to the petri dish and the dish was incubated for 10 minutes. The DMEM was removed and the coverslip was washed with DMEM once. Growth medium was added and the coverslip was transferred to the observation chamber. This process was repeated once, images were captured on the same day as the coverslip treatment.

### *Experimental: High Dose*

The petri dish containing the cover slip in growth medium was removed from the incubator and the growth medium was discarded. 500 ml DMEM and 10 ml 236 uM Mitotracker were added to the petri dish and the dish was incubated for 10 minutes. The DMEM and Mitotracker solution was then removed and the petri dish containing the coverslip was washed with DMEM three times. 40 nM MeHg DMEM was then added based on the concentration used in Leong's "Retrograde degeneration of neurite membrane structural integrity of nerve growth cones following in vitro exposure to mercury" but modified for whole culture incubation conditions. This was then incubated for 10 minutes, at which time the MeHg DMEM solution was removed. The coverslip was washed with DMEM once, and growth medium was added. The coverslip was then transferred to an observation chamber. This process was done one day; images were captured the same day

### *Experimental: Low Dose*

The petri dish containing the cover slip in growth medium was taken from the incubator and growth medium was removed. 500 ml DMEM and 10 ml 236 uM Mitotracker was added to the dish, which was then incubated for 10 minutes. The DMEM and Mitotracker solution was then removed and the coverslip was washed with DMEM 3 times. 20 nM MeHg DMEM was added, based on the concentration used in Leong's "Retrograde degeneration of neurite membrane structural integrity of nerve growth cones following in vitro exposure to mercury" but modified for whole culture incubation conditions. The petri dish containing the coverslip was then incubated 10 minutes. The MeHg DMEM solution was removed from the dish and the coverslip was washed with DMEM once. Growth medium was

added and the coverslip was transferred to an observation chamber. This process was done one day, images were captured the same day.

### *Viewing the Cells*

Slides were viewed and images were taken in the ICUC using a computer named “Gemini.” The microscope was set with Koehler illumination and the correct focal plane was found using the 10X objective lens. Cells were then located and the objective lens was changed to 40X, at which time glial cells were found (Morris, 2014). Pictures were taken using Nikon E-200 microscope with Spot camera 1X with Spot software. The light source was then blocked and the microscope was switched to the red emission filter. The fluorescence shutter was opened and a picture was taken using the Spot camera. Typical exposure times were 1-3 seconds. Pictures were taken in collaboration with Anya Sokolova.

### *Analysis*

The fluorescent pictures taken were analyzed using ImageJ. Mitochondria could be seen as bright red spots on the image. Measurements were taken to the brightest part of the mitochondria, which was typically the center. The distance of the six farthest mitochondria from the center of the nucleus and the width and length of the glial cell were measured using the measuring tool. The length was considered the long axis of the glial cell and the width was the shorter axis. The long axis was the longest linear distance between two points of the glial cell boundary. The distances were normalized by calculating the percent of the total length or width of the glial cell the distance of mitochondria was. If a mitochondrion was measured exactly on the short axis, they were calculated as a percent of the total width. This led to two mitochondrial distances being normalized by width per cell. The four other mitochondrial distances in each cell were measured as a percent of the total length. Measurements were taken in collaboration with Anya Sokolova. The values for 4 control glial cells were averaged, as were values for the 6 cells treated with a low dose of MeHg and the 3 glial cells treated with the high dose of MeHg. The controls were necessary to determine if methyl mercury caused a change in the dispersion of mitochondria in the glial cells. The control is appropriate because the only difference between its methods and the experimental methods is the presence of methyl mercury in one step. All other variables were kept the same. Different doses of methyl mercury were used to see if the distribution of mitochondria within the glial cells changed when in the presence of varying amounts of methyl mercury.

### **Results:**

A single glial cell was seen in the control slide. Figure 1 shows one of the glial cells that were observed. Mitochondria were observed throughout the cell, which can be seen as the bright red areas within the cell. The mitochondrial distance away from the center of the nucleus was measured as a percentage of the total cell length. This was calculated by dividing the mitochondria distance from the nucleus by the total length or width of the glial cell. The width was considered to be the short axis and the length, the long axis of the glial cell. All distances were normalized as a percent of the long axis length with the exception of the two measurements from the nucleus to the mitochondria directly on the short axis on either side of the nucleus. The averaged control glial cells percent distance was 36.1%. Figure 2 displays a glial cell introduced to 20 nM of MeHg. Mitochondria were seen throughout the cell. The average distance the mitochondria were from the nucleus was 37.7% of the total length. Figure 3 shows a glial cell treated with 40 nM of MeHg. Mitochondria were seen throughout the cell. The mitochondria were an average distance of 32.2% of the total length away from the mitochondria. The number of glia that were observed varied with an N-value of 4, 6 and 3, respectively. Figure 4 conveys a graph comparing the percent distance traveled for all 3 groups. There was no observable difference between control and experimental values.



Figure 1. An example of a glial cell. The black line indicates the length, the long axis. The red line indicates the width. Picture was taken in collaboration with Anya Sokolova.

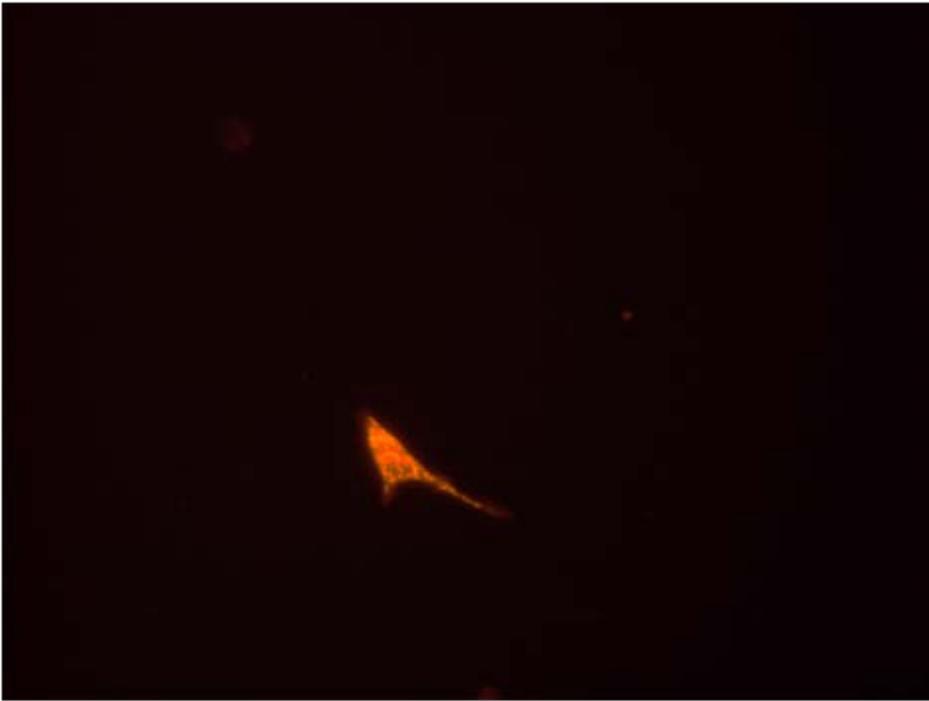


Figure 2. An example of a glial cell. Mitochondria are distributed throughout the cell, with a high concentration near the nucleus. Picture was taken in collaboration with Anya Sokolova.



Figure 3. An example of a glial cell treated with 20nM MeHg. The black line shows the length. The glial cell has long processes along the axis indicated by the black line. The red line shows the width. Picture was taken in collaboration with Anya Sokolova.

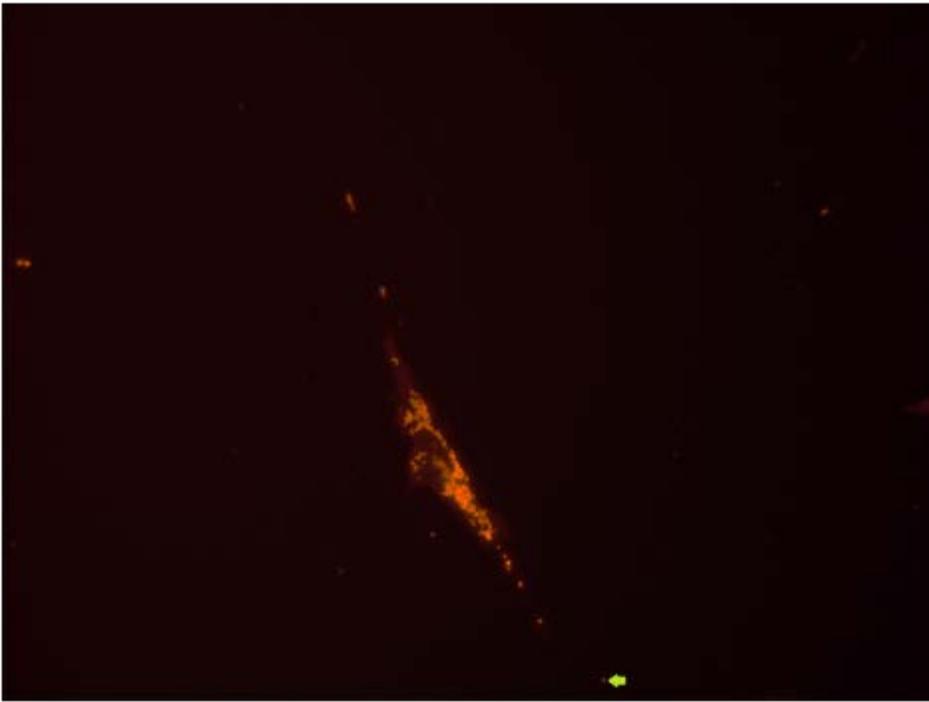


Figure 4. An example of a glial cell treated with 20nM MeHg. Mitochondria can be seen throughout the cell. Individual mitochondria can be seen far down the processes of the cell. The green arrow at the bottom right of the image indicates the farthest mitochondria down that process. Picture was taken in collaboration with Anya Sokolova.



Figure 5. An example of a glial cell treated with 40nM. Mitochondria can be seen throughout the cell, with high

concentrations in the processes. Picture was taken in collaboration with Anya Sokolova.

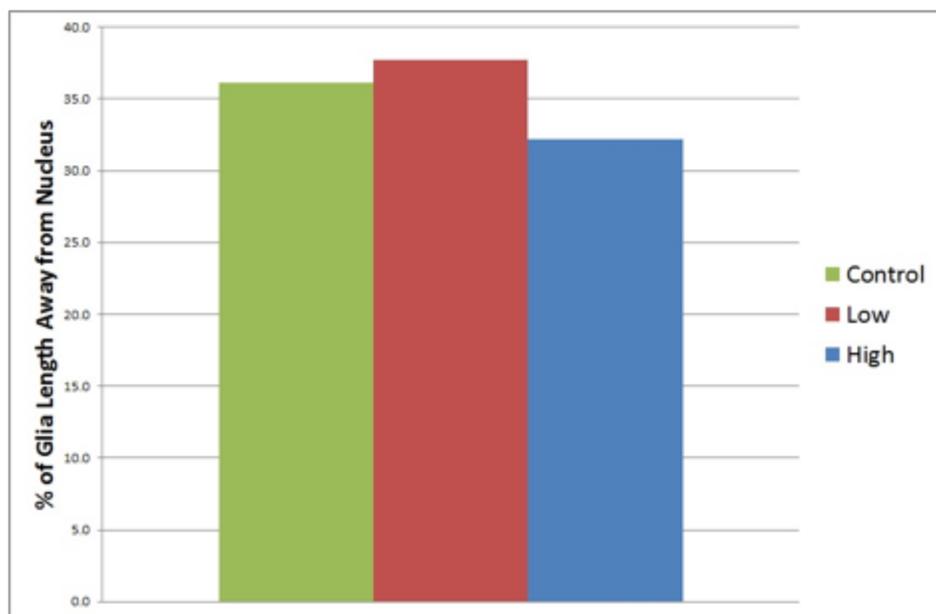


Figure 6. The average percent of total cell length away from the nucleus of mitochondria in glial cells. There was no obvious trend between control and experimental glial cells given the small sample sizes.

## Discussions and Conclusions:

In this study we tested the hypothesis that in the presence of increasing amounts of methyl mercury, the mitochondria of the glial cells will move farther from the nucleus. These results do not support this hypothesis. There was no obvious difference between experimental and control values, given the small sample size (Figure 4). Glial cells treated with methyl mercury did not have their mitochondria move a farther distance away from the nucleus than the control cells. The mitochondria in the high dose experimental group were actually slightly closer to the nucleus than the control group. The inconclusive data could be explained by the slow sample size for the control, low dose and high dose groups (n=4, n=6, n=3, respectively).

Upon further research, it was also found that damaged mitochondria are transported back to the cell body to be degraded or replenished. This explains the difference in behavior of what was originally expected of the mitochondria. The mitochondria are transported back to the cell body because the cell body is abundant in lysosomes, ribosomes and other organelles. Because of this, although neurons need to distribute the mitochondria down processes for energy, it is also important that they are able to get rid of them when necessary (Lovas & Wang, 2012). This information supports

the trend found that the mitochondria in the high dose experimental group were closer to the nucleus than the control group. The damaged mitochondria traveled back towards the nucleus to be replenished or to be degraded. Future work should be done using a new hypothesis that if glial cells are treated with increasing amounts of methyl mercury, that the mitochondria will move closer to the nucleus. This should be done using larger sample sizes and increased concentrations of methyl mercury.

Sources of error could have occurred from the cells becoming dried out during changing of buffers, or during the creation of the observation chamber. Glial cells becoming dry damages the cells, which could lead to differences in the reactions of the cells to methyl mercury.

If the data had been found as a result of one thousand replicated experiments, the researcher would speculate that the calcium channels were not inhibited by the 20um dose of methyl mercury, which is why no obvious change is seen between the low dose and the control, but that they were inhibited by the increased concentration of 40 um methyl mercury. The decrease in distance of the mitochondria from the nucleus seen in Figure 4 could that the calcium channels were inhibited by methyl mercury, and the mitochondria were damaged by it also. These conditions may have led the damaged mitochondria to be able to move back to the cell body where they could be replenished or degraded.

It could also be possible that the concentrations of methyl mercury were not high enough. Methyl mercury irreversibly suppresses calcium entry (Atchison & Hare, 1994). At resting calcium levels, mitochondria are able to move freely throughout the cell. However, when the cytosolic calcium levels increase, the mitochondria are inhibited from movement to increase ATP and buffer the calcium concentration (Lovas & Wang, 2012). If the doses of methyl mercury were not high enough, the calcium channels would not be inhibited, and the mitochondria would not be able to move in the glial cell. This could be why there is no obvious change between the mitochondrial distance from the nucleus for the control and low dose groups. The small difference between the control and high dose groups could be because there was a high enough concentration of methyl mercury to inhibit the calcium channels and allow more mitochondrial movement. If the experiment were to be repeated, 40uM would be used as the low dose and 80uM as the high dose.

## **References:**

Atchison, W. D., & Hare, M. F. (1994). Mechanisms of methylmercury-induced neurotoxicity.

Hollenbeck, P. J., & Morris, R. L. (2014). Primary culture of chick embryonic peripheral neurons

1: Dissection. In

Kandel, E. R., Schwartz, J. H., Jessell, T. M., Siegelbaum, S. A., & Hudspeth, A. J. (2000).

Principles of neural science. (5 ed.).

Leong, C. C. W., Syed, N. I., & Lorscheider, F. L. (2000). Retrograde degeneration of neurite

membrane structural integrity of nerve growth cones following in vitro exposure to mercury. *Membrane Biophysics and Biochemistry*, 12(4), 733-737.

Lovas, J. R., & Wang, X. (2012). The meaning of mitochondrial movement to a neuron's life.

*Biochimica et Biophysica Acta- molecular cell research*, 1833(1), 184-194.

Martini, F. H., & Bartholomew, E. F. (2007). *Essentials of anatomy and physiology*. (6th ed.).

Pearson.

Michaud, M. Support cells found in human brain make mice smarter. (2013). University of

Rochester Medical Center, Retrieved from <http://www.urmc.rochester.edu/news/story/index.cfm?id=3770>

Morris, R. L. (2014). Primary culture of chick embryonic peripheral neurons 2: observation of

live unlabeled cells. In

Sokolova, A. (2014).

United States Environmental Protection Agency, (2012). Methylmercury. Retrieved from

website: <http://www.epa.gov/hg/effects.htm>

Yin, Z., Milatovic, D., Aschner, J. L., Syversen, T., Rocha, J. B. T., Souze, D. O., Sidoryk, M., Albrecht, J., & Aschner,

M. (2007). Methylmercury induces oxidative injury, alterations in

permeability and glutamine transport in cultured astrocytes. *Brain Research*, 1331, 1-10.

