Analysis of Mitochondria in Glial Cells: Methyl Mercury Effect on Mitochondria Distribution in Glial Cells

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Introduction

The central nervous system is made up of neurons and glial cells (Kandel, 2013). The glial cells have a diverge range of functions including: insulation, ion concentration regulation, and growth factor release (Kandel, 2013). In an embryo, glial cells are responsible for forming a framework that permits the development of the nervous system (Jessen, 2013). They are also responsible for providing nourishment, insulating synapses, and most importantly helping accelerate the conduction of neuronal signaling (Jessen, 2013). Mitochondria in glial cells are also the main focus of this research. Since mitochondria are the location of ATP synthesis, it is important to study their distribution throughout the glial cells. Energy is the most important aspect of a cell, and being the source of this energy makes is very important to study mitochondria. The function of glial cells is energy dependent, and studying the distribution of mitochondria will help with understanding how their normal function is affected. This study is important for also other mitochondria related disorders and their association with methyl mercury.

Mercury is a well-known environmental toxin that is spread throughout nature and implicated in a range of neurological and psychiatric disorders (Ni, 2011). Mercury causes severe damage to the central nervous system, especially during development. There are various disorders caused by mercury that can impact humans' life, such as: mental retardation, dysarthria, growth disorder, hyper salivation, etc (Ni, 2011). Studies have shown that mercury has a

large effect on neurons and their axons, but there are not many studies done on their effect in glial cells (Eskes et al., 2002). Since the developing central nervous system is much sensitive to mercury poisoning, it is suggested that the glial cells are very vulnerable to mercury poisoning, since the glial cells play a big role in forming the nervous system. There is strong evidence that mercury exposure causes a distribution in membrane integrity of neuronal growth cones, which may implicate mercury as a potential factor in neurodegeneration (Leong et al, 2001). Based on this existing research, it can be suggested that methyl mercury has the same effect on glial cells as well. This was a motivation to start studying this aspect of the central nervous system.

In the present study we tested the hypothesis that if exposed to methyl mercury, then the mitochondrial location within the glial cell will differ by retracting back towards the centroid. The model used for this study was an in vitro exposure of methyl mercury on mitochondria of glial cells in growing peripheral chicken (*Gallus gallus*) neuron culture. The reason why this species was chosen and used is because of the similarities between the chick's nervous system and to that of the human species. Using fluorescent microscopy, the mitochondria of the cultured glial cells were identified and then their distance from the centroid was measured and quantified using the program Image J. This research is important because there have been no other studies found on the effect of methyl mercury on the distribution of mitochondria in glial cells. In Hollenbeck's research in 2013, the movement of mitochondria and positioning in axons was studied, but there is no information found on how methyl mercury effects the distribution of mitochondria in glial cells (Hollenbeck and Chada, 2003). Studying this effect will help the scientists to better understand the impact and implications that this toxin has. This is also very important because glial cells hold essential roles in our nervous system.

Materials and Methods

Embryo Explanting:

For this experiment, domestic chicken embryos were dissected and the dissection procedure was done based on Peter J. Hollenbeck protocol with only a few changes (Morris, Dissection, 2014). The data sets collected were always in pairs: one control and one experimental. On the control data, there was no methyl mercury exposure, where in the experimental data, the cells were exposed to a low dose of methyl mercury. Neurons in this experiment were plated for 38 hours instead of 24 hours giving more time to nerves to grow. 60 ganglia and 6 sympathetic nerve chains were plated which increased the concentration of our neurons in the petri dishes. This gave us a bigger number of axons to study. The coverslips were left on poly-lysine treatment for 3 hours instead of 20 minutes to increase the adhesiveness of the

neurons on the petri dishes. In order to increase the signal of growth, the coverslips were left over laminin treatment for 2 hours. Other changes done to the dissection procedure are: increase of the nerve growth factor from 50 ng/mL to 200 ng/mL for a better growth signaling in the growth medium; and an increase in the glutamine concentration from 2mM to 4 mM to prolong its time in the medium and avoid its break down. Another technique used to help with the neuron adhesion was the isolation of incubator's shelves from vibrating.

Fluorescent Staining:

Vital-stain fluorescence microscopy technique was used to stain the live cells using the protocol provided by Doctor Robert Morris (Morris, StainAndObserv, 2014). After multiple dilutions, the concentration of the mitotracker was changed to 118nM. A flow chamber was assembled in order to flow solution through the nerves (in our case: DMEM for control or DMEM + methyl mercury for experimental). For the flow chambers, only the two sides of the cover slips were sealed, so that we could flow solutions through the glass and cover slips. Fluorescence pictures of the nerves were taken using a Nikon Eclipse, Spot InSight Firewire 2 Megasample camera, and the Spot program on a Macintosh. The exposure time to fluorescence light was about 520 milliseconds. The objective lens used was 40x. After the control data were collected, experimental data with 4nM (low concentration) methyl mercury exposure were also conducted. On prior tries, 40 nM methyl mercury exposures were studied as a high concentration data. The experimental data were collected in the exact same circumstances as the control data, in order to find out any differences on the distribution of mitochondria in glial cells when exposed to methyl mercury. A heater was also used during the data collection, in order to keep the cells warm at 37^{o}C and alive.

Measurements:

The distance of mitochondria from the centroid (center of glial cells) was measured using the program ImageJ. The centroid was identified approximately, using the measuring tools from ImageJ to find the center of the glial cells. Using the straight line/measure tool, I studied how does methyl mercury effect the distribution of mitochondria along the glial cell. The mitochondria chosen were random, bright, distinctive, and measurable. The distance was measured from the middle of the mitochondria to the centroid of the glial cells. There were two glial cells studied for this experiment, and ten mitochondria. The straight-line tool gave us the lengths in pixels, which were converted to μm using the bar scale method. The mean value of these measurements was calculated and a bar graph was generated with standard deviations

shown.

Results

The data represent the distribution of ten different mitochondria in glial cells. Ten different data sets were collected including measurements from the control with no methyl mercury exposure and the experimental with a low dosage of methyl mercury exposure. On figure one, two glial cells were recorded using light microscopy and the arrows indicate the location of the mitochondria studied. These same glial cells were used for the control and the experimental data.

Figure1. Image of control taken with light microscopy. Arrows highlight the mitochondria studied in the experiment. The distribution of mitochondria is random, but distinguishable.

The control figure (Figure 2) shows the distribution of mitochondria in the glial cell after the cells were stained.

For this figure the flow chamber technique was used to flow DMEM through the glial cells. The experimental picture (Figure 3) shows the effect of methyl mercury on these same mitochondria identified on Figure 1 and Figure 2. As mentioned on the Materials and Methods section, the dosage of methyl mercury used for this experiment was very low: 4mM. The images for the experimental data were taken 5-7 minutes post methyl mercury exposure. On the first and second picture, ten mitochondria were located on the glial cell and their movement was studied. On the third picture, movement of these mitochondria towards the centroid were observed and recorded. The distances were measured and reported on Figure 4. A decrease of the mitochondria distance from the centroid was observed when the cells were exposed to methyl mercury.

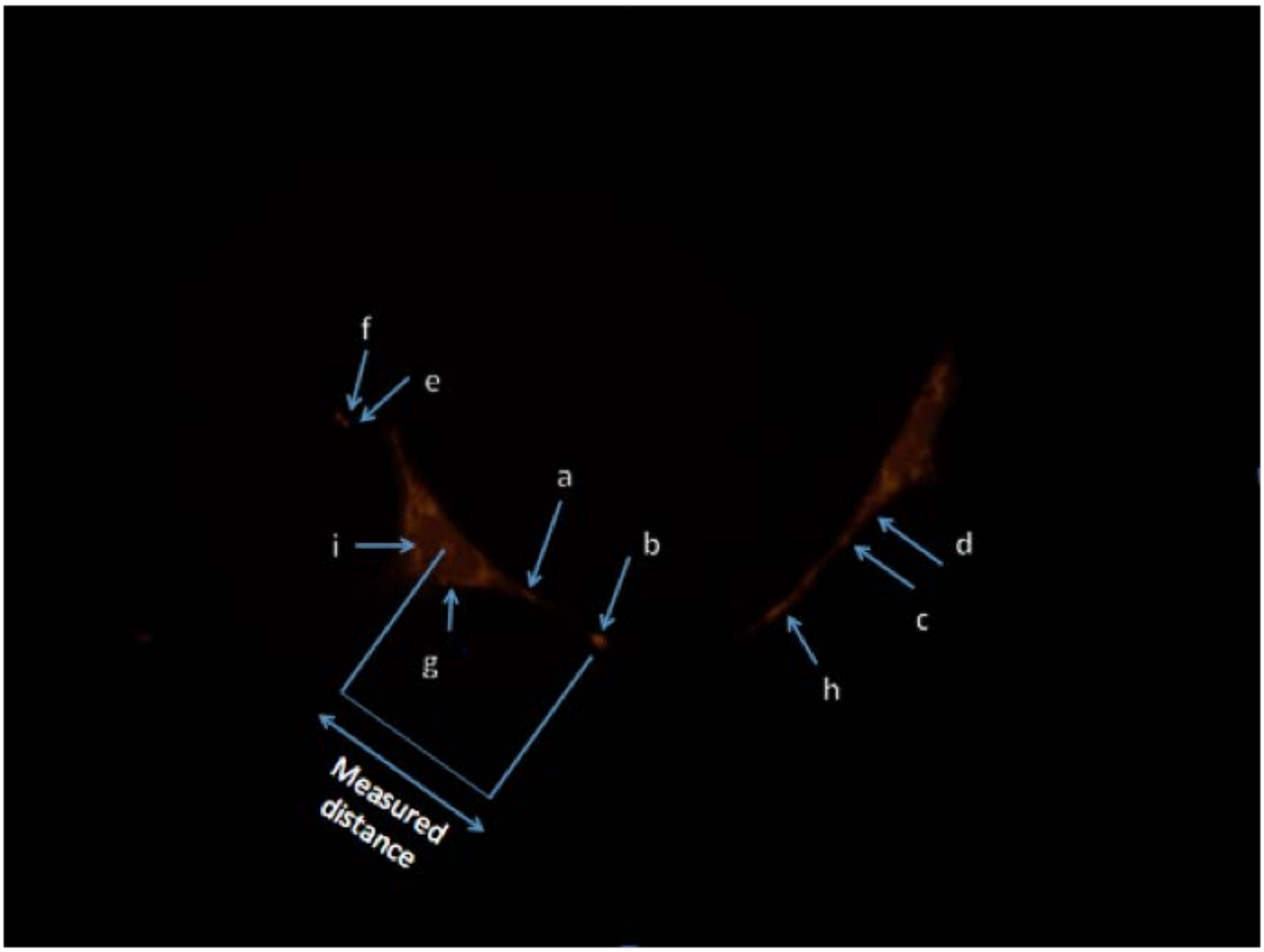

Figure 2. Control data: Image of the same mitochondria (as located on Figure 1). A mitotracker of concentration 4 nM was used to stain the cells and then fluorescent microscopy was used to take pictures. This picture was taken after DMEM was flowed under the coverslip so we can keep the control with experimental data under the same conditions. The bracket shows how the distances were measured. In this specific example, it is shown how it was measured from mitochondria "b" to the centroid and the result was recorded in μm.

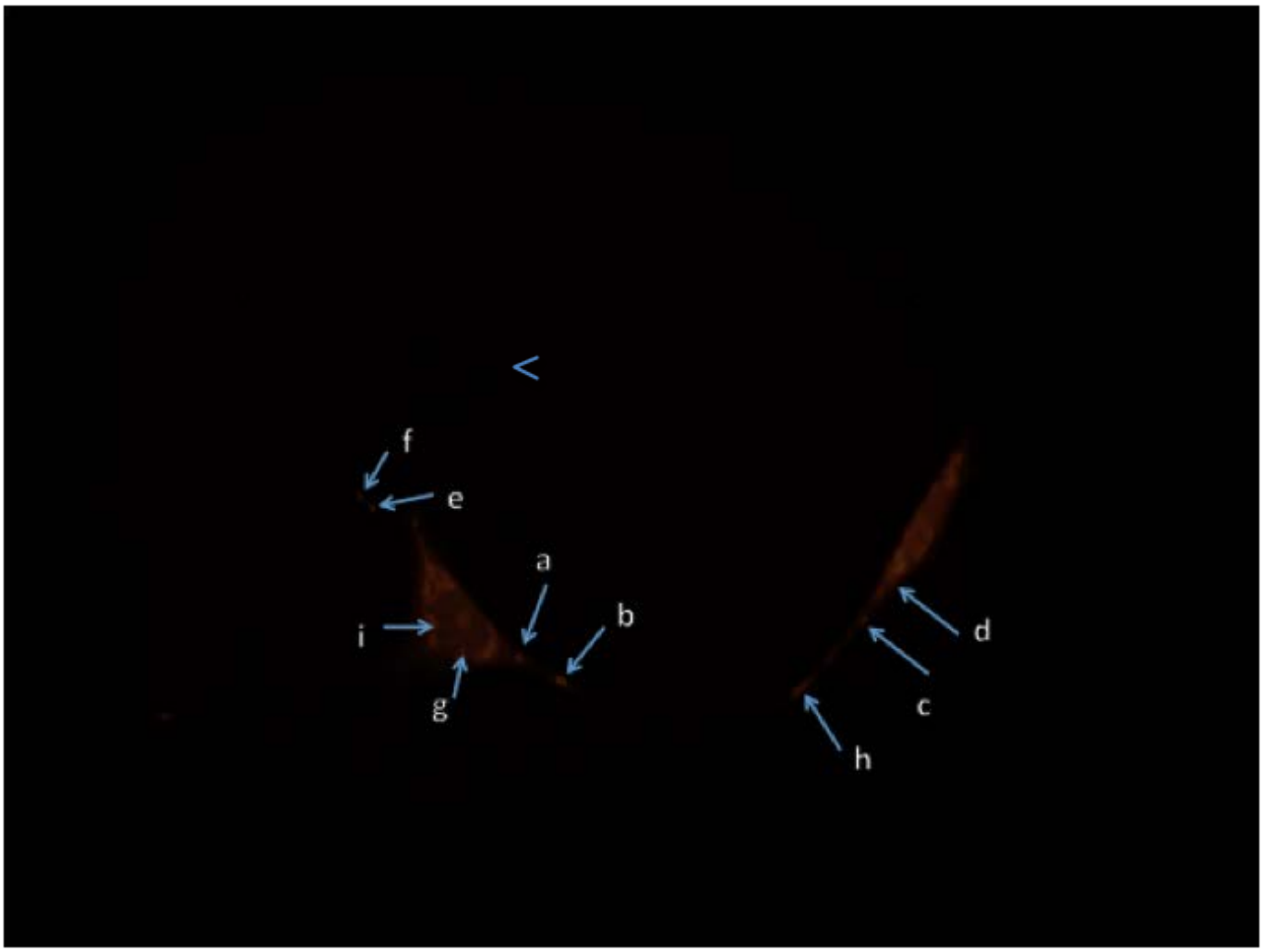

Figure 3. Experimental Data: Image of the same mitochondria (as located on Figure 1 and 2). A mitotracker of concentration 4 nM was used to stain the cells and then fluorescent microscopy was used to take pictures. This picture was taken after methyl mercury and DMEM was flowed under the coverslip so we can keep the control with experimental data under the same conditions. If compared with picture 2, here it is shown the movement of mitochondria towards the centroid.

The distances were recorded in micrometers and Figure 4 above shows the means of these generated data. Mitochondria 1 for instance (labeled as “a” in the pictures) had a distance of 20μm from the centroid under normal conditions. When the glial cells were exposed to methyl mercury, this distance decreased to 4μm. This shows a change of the distance by a factor of five.

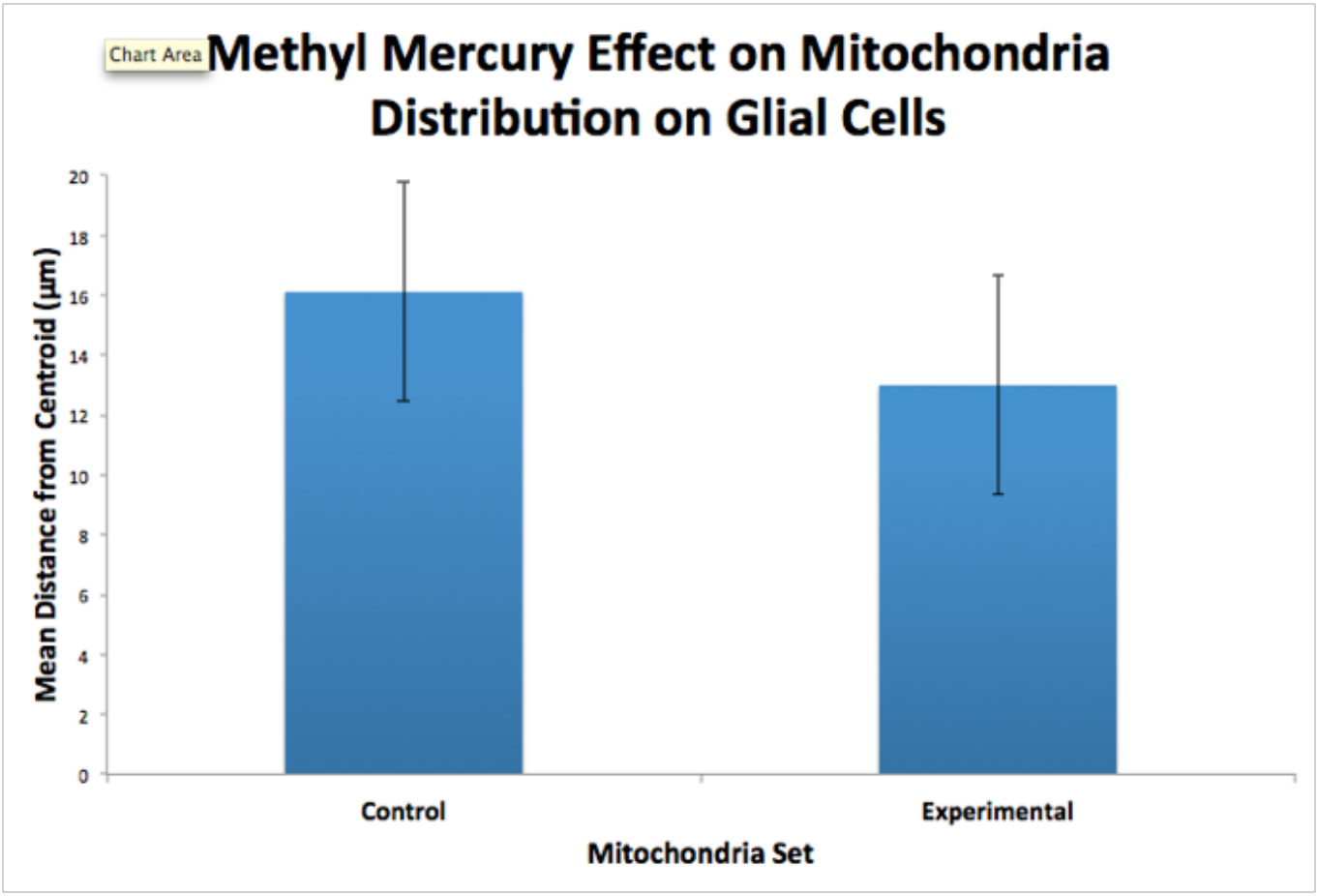

Figure 4. The Image J results are reported on this graph. The mean distances of mitochondria from the centroid were averaged, and the standard deviations are also shown. The total number of mitochondria studies was **ten**. The bar graphs show that there is a decrease in the distances of the mitochondria from the centroid after being exposed to methyl mercury.

Discussion and Conclusions

The hypothesis of my experiment was supported, because as shown on Figure 4 when the glial cells were exposed to the low dosage of methyl mercury, the distance from the centroid decreased. For almost all the mitochondria observed, this distance after methyl mercury exposure was a quarter less the original distance recorded. These changes suggest and support that methyl mercury has a large impact on mitochondria distribution in glial cells as my hypothesis stated.

These results are very important understanding the origin of many disorders identified in previous studies, which originate from mercury exposure. The fact that the data suggest that the mitochondria in glial cells, when exposed to methyl mercury, start retracting back towards the centroid, can be connected with the growth disorders or problems with coordination. As mentioned above in the introduction, glial cells play an important role in neural growth and in

conductance of neural signals (Jessen, 2004). So if methyl mercury is introduced to these cells, effecting the mitochondria distribution throughout the glial cells, then it makes sense to expect these malfunctions in the nervous system, originating from the glial cells.

The n value for this study was reasonable, but a higher number of mitochondria studied is suggested. Even though most of the ten mitochondria supported my hypothesis, there is always space of improvements and for more accurate data results by observing a larger quantity of mitochondria. This was not done in this experiment, because a limited number of glial cells were captured during photography. If that were done, and if the same results were collected from these data, then my hypothesis would have had a much stronger support and been more accurate. If there were a large amount of data enough that I were convinced that all the trends and differences between experimentals and controls were real, then this would have been very beneficial for a further analysis on the molecular level. As introduced before, mercury exposure to neurons causes multiple diseases, and by studying how the glial cells are affected, we would be able to connect these results with particular diseases (Ni, 2011). Glial cells are responsible for forming the framework for the development of the rest of the nervous system (Jessen, 2004). Thus, it is most likely that the conduction of neural signaling would decrease when exposed to mercury.

Further studies should be done on this subject, where glial cells are exposed to a bigger variety of methyl mercury concentrations. This would assist with identifying how much methyl mercury exposure can be endured. These results can help the scientists with future medical applications or studies on environmental toxins such as mercury. Along this, scientist can use this data to determine the amount of mercury exposure that causes significant damage to the nervous system. Another experiment that could be conducted in the future is prolonging the mercury exposure and observing the effects of it to glial cells. This experiment can show us if methyl mercury has lethal effects on the cells. Different doses of methyl mercury can also be used in future experiments in order to find the minimal amount of methyl mercury concentration that causes an effect on neurons, as well as how are these different dosages effecting them.

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