

Decreased Mitochondrial Activity in Glial Cells of Peripheral Neurons after *In Vitro* Exposure to Mercury

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Introduction:

There is growing evidence to support that mercuric chloride is a neurotoxin which degrades microtubule structure in neurons of the central nervous system (Leong et al. 2001). In previous studies (Leong et al. 2001) 77% of neurons subjected to *in vitro* exposure to mercury had disturbed linear growth rates and membrane structure. The rising interest on the effects of mercury at the cellular level, can be partially attributed to accumulating evidence that chronic exposure to mercury vapor released from dental amalgam tooth fillings may have undesired neurobehavioral effects (Leong et al. 2001).

Mercury has not only been shown to affect the structural integrity of neurons, but also to have an effect on the glial cells that support and regulate the activity of neurons. Mercury as well as other neurotoxic metals have been shown to collect in glial cells and may play a role in triggering a cascade of degeneration and apoptosis (cell death) in surrounding neurons (Toimela and Tahti 2004). The apoptosis cascade is known to be regulated by mitochondria, and is hypothesized to be triggered by detrimental fluctuations in mitochondria membrane potential (Toimela and Tahti 2004). Although glial cells may show effects of mercury exposure more rapidly than neurons (Toimela and Tahti 2004) they have also been shown to be less sensitive over time, with less accumulated effects than neurons (Kaur, Aschner and Syversen 2006).

The present study tests the effects of *in vitro* exposure to mercuric chloride on mitochondria of glial cells in growing peripheral chicken (*Gallus gallus*) neuron culture. *Gallus gallus* was used in this experiment because of the similarity between the nervous system of this species to that of the human species. The density of mitochondria was studied in control glial cells with no exposure and in a group of experimental glial cells which received exposure to mercuric chloride. The mitochondria of the cultured glial cells were imaged using rhodimine 123 (R123) vital dye. R123 is a popular dye used for mitochondria because it is known to be non-invasive and sensitive, responding to slight changes in membrane potential and reflecting this in florescence (Huang et al. 2007). It is hypothesized that the results of this

experiment will show decreased mitochondrial activity in mercury exposed glial cells, and therefore less brightness produced from the R123 dye in comparison to unexposed glial cells. The observation of these changes would be significant because according to the research described above the alteration of mitochondrial activity could be significant in regulation of the negative structural changes and apoptosis observed with mercury exposure (Toimela and Tahti 2004).

Materials and Methods:

Materials and Equipment

Materials for coverslip cleaning as well as the materials required for the primary culture of neurons, please refer to laboratory handout: *Primary Culture of Chick Embryonic Peripheral Neurons* provided by Morris (2011a). In addition to these materials mercuric chloride ($10\mu\text{m HgCl}_2$ in .05% HCL) and Rhodamine 123 vital dye (.50mg/ml for 10 minutes incubation time) were used in this experiment. Cells were viewed, and images were taken using a E200 Nikon Eclipse Microscope with phase optics and florescent light capabilities with a Sony DFW – x700 digital interface camera. This digital interface camera has a C- mount 1x. The computer used in this experiment was an iMac OS x version 10.5.8 with BTV version software. Image J photo analysis software was used in this experiment to measure the intensity of fluorescence created by the R123 on the mitochondria in the glial cells.

Methods:

Detailed steps of the procedure for the initial culturing of neurons for this experiment can be found in: *Primary Culture of Chick Embryonic Peripheral Neurons 1. Dissection* (Morris 2011a). Before culturing the neurons, coverslips were cleaned and prepared, the pasture pipettes were flame-constricted and the coverslips were treated. The initial steps of dissection were carried out, gathering the embryonic tissue of the chick embryos and was continued until peripheral nerve chains and dorsal root ganglia were obtained. Coverslip treatments continued during the dissection process. Cells were incubated at 37° Celsius. After the incubation period (a duration of at least 17 hours) cell growth was viewed following the steps described in: *Primary culture of Chick Embryonic Peripheral Neurons 2. Observation* (Morris 2011b).

These steps described were repeated with the addition of a flow chamber to collect data on the effects of flow. The flow chamber was created using glass chips and a VALAP seal, and HBSS/ Hanks solution was flowed through the chamber to show the effects flow has on the neurons and glial cells without mercury.

For a detailed description of the steps taken to stain the cells with R123 and view under fluorescence please refer to:

Neurons 3. Staining and Observation (Morris 2011c). Peripheral Neurons were stained with rhodamine 123 and six images were taken of these glial cells in phase and six more images in fluorescence. The fluorescent images were captured using a two second exposure setting. These images served as the images for control data collection. Mercury was then flowed through the flow chamber created in the steps above. The mercury was left in the flow chamber for 20 minutes and then HBSS was passed through the flow chamber. Six images of cells were taken after this mercury treatment in phase and fluorescence (same exposure for images). All images for the control and experimental group were taken immediately following flow, thus limiting the time available of regeneration of cells after mercury exposure.

Data Quantification:

1. Image J was used to open the florescent and phase images of glial cells and neurons
2. Without adjusting the image brightness properties, the glial cells were located. The glial cells were identified using the shape characteristics of glial cells. Axons of neurons were classified as linear structures which often converge and form branch points (which appear smaller than most glial cells). The glial cells were defined as triangular shaped areas of brightness which has a distinguishable nucleus. See Figure 1 for a description and visual example of a well defined glial cell.
- Depending on the clarity of fluorescence defining and measuring glial cells required zooming in on the image and comparing the fluorescent and phase images (using fixed aspects of the photo for reference points). Some images were not used because they contained a single axon or growth cones and had no distinguished glial cells. This created an unequal number of photos and cells used in each group of data.
3. Four control images were used, two flow images were used and six mercury images were used, all with varying number of usable cells.
4. Each glial cell was outlined (only used those which could be clearly identified and had a distinguishable shape) using the tracing freehand tool.
5. After a border was drawn around the cell the average brightness of the cell was measured through the program options (brightness of pixels).
6. These values were recorded for each glial cell in the control, flow control and mercury exposed groups.
7. The average brightness values were then averaged within each group and compiled into a table and graph using Microsoft Office Excel.

Results:

While finding the glial cells in the images of each group of data, it was observed that there were images from each

group (most significantly the control) in which there were no usable glial cells. Because some images were not usable and the number of cells from each picture varied, there were a different number of cells quantified for each group. n=24 for the experimental group (24 cells measured from 6 images), n=20 for the control data (from 4 images), n=11 for the flow images (from 2 images).

The average brightness from each group (totaled brightness from each photo in a group) is recorded in figure 3.

Although both groups did not receive mercury, the data for the control and the flow groups cannot be combined because there is a possibility that the flow could create a difference in the brightness and mitochondria activity in the glial cells.

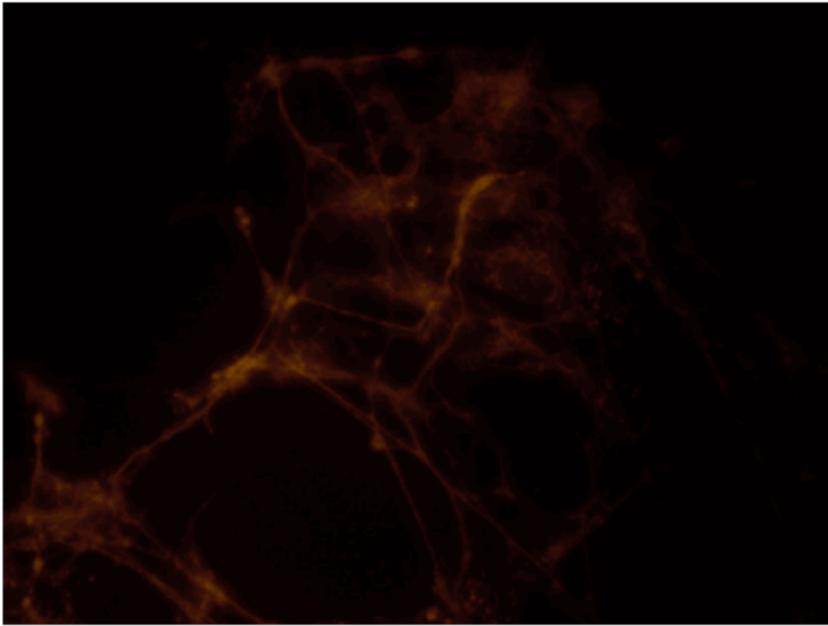


Figure 1: This figure shows the fluorescence of the glial cells (The R123 shows the positioning of mitochondria in the cells) from an experimental group. This means that the cells in this image have received mercury treatment. Observe the cluster of glial cells surrounded by neurons in the center of the image. An example of a well defined glial cell is C-shaped and can be found near the most fluorescent axon in the center of the image (the open face of the C is facing down).

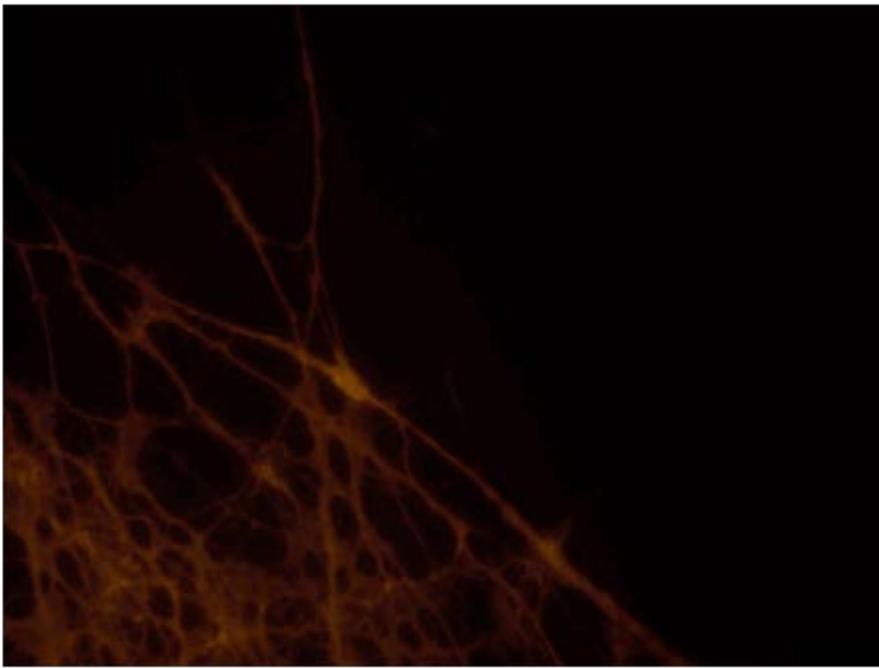


Figure 2 – This image was selected from the control group. The cells in this image are fluorescent with R123 dye and have received no mercury exposure. Note the abundance of glial cells in the lower left quadrant. During data collection this area was magnified to take specific cell fluorescence measurements. Observe the increase in fluorescence of the glial cells in this control image in comparison to the glial cells in the experimental image above.

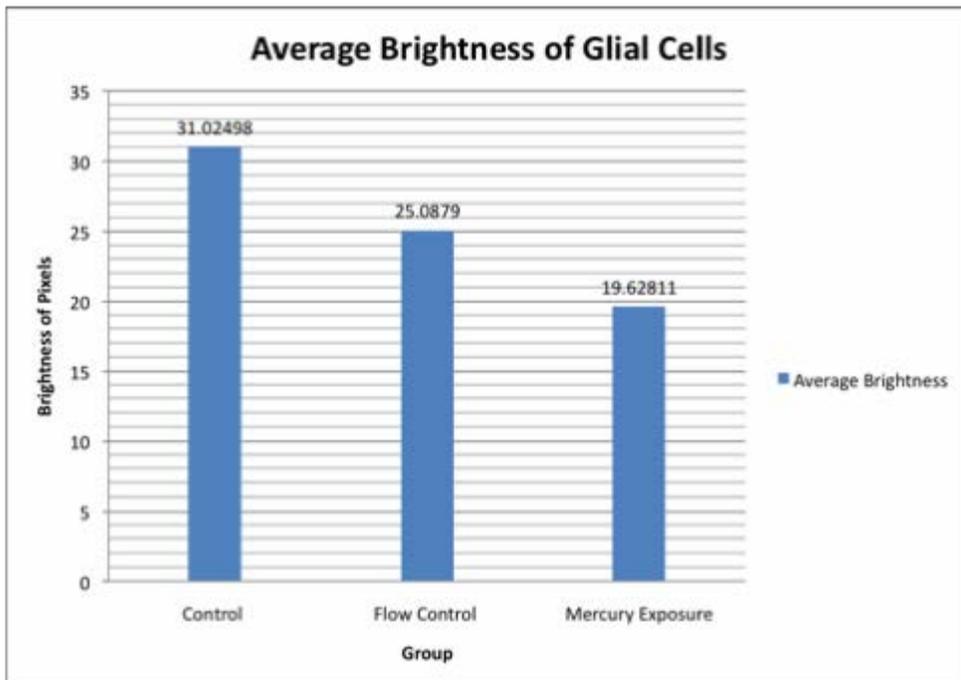


Figure 3: This figure demonstrates the difference in the average brightness of glial cells in the control group, flow group (which received only HBSS) and experimental group (which received mercury treatment) when stained with R123. The mercury group has the lowest average brightness, demonstrating that brightness of glial cells and therefore

mitochondrial activity decreases when exposed to mercury.

Discussion:

The data collected from this experiment support the hypothesis that the chick peripheral embryonic glial cells which were exposed to mercury have lower brightness values than glial cells which are not exposed to mercury. The discrepancy between the brightness values of the two groups may represent the effects of mercury on the mitochondria of glial cells. Because mitochondria are producing the membrane potential that R123 responds to (illuminates), the difference in fluorescence and brightness of the glial cells represents the change in mitochondrial activity. Decreased mitochondrial activity and therefore decreased brightness could be the result of decreased membrane charge in the mitochondria. As proposed by Toimela and Tahti in 2004, this change in membrane charge could have a significant influencing factor over the apoptosis or structural degradation that occurs in the neurons surrounding the glial cells. Though the results of this experiment support this theory, only with a larger pool of data and repeated experiments could it be concluded that *in vitro* mercury exposure causes a decrease in mitochondrial activity of glial cells.

During the collection of data it was observed that the experiment could be improved a few points. First, pictures for this experiment should be made focusing on glial cells and secondly, the outline of each glial cell would be made more accurate by overlaying the phase and fluorescent images, or somehow better defining their membrane borders.

Although each glial cell may have been traced slightly inaccurately in this experiment, the error should be even in the experimental and control group, therefore not affecting the difference in brightness between the two groups. More data should also be collected on the effects of flow on glial cell brightness. The average brightness of the glial cells which received flow in this experiment were slightly lower than the controls, and this difference is hypothesized to be insignificant however more data to support this claim needs to be collected.

Future experiments should be carried out not only testing this brightness hypothesis, but possibly altering it to count the specific number of mitochondria or follow their position in the glial cell after mercury exposure (using phase images).

Further experiments should also be conducted focusing on testing other aspects of the theory presented by Toimela and Tahti in their 2004 paper that the mitochondria of glial cells play such a large role in monitoring the effects of mercury, such as apoptosis, on surrounding neurons.

References:

Chadwick, E.H., Tratner, V., Pauly-Hubbard, H., (2011). Personal Communication.

- Morris, R.L. (2011a) Neurobiology Bio324 Primary Culture Of Chick Embryonic Peripheral Neurons 1: DISSECTION. Available at <http://icuc.wheatonma.edu/bio324/2011/LabProjProc/1.htm>.
- Morris, R.L. (2011b) Neurobiology Bio324 Primary Culture Of Chick Embryonic Peripheral Neurons 2: OBSERVATION. Available at <http://icuc.wheatonma.edu/bio324/2011/LabProjProc/2.htm> .
- Morris, R.L. (2011c) Neurobiology Bio324 Primary Culture Of Chick Embryonic Peripheral Neurons 3: STAINING and OBSERVATION. Available at <http://icuc.wheatonma.edu/bio324/2011/LabProjProc/3.htm>.
- Huang, M., A. K. S. Camara, D. F. Stowe, F. Qi, and D. A. Beard. "Mitochondrial Inner Membrane Electrophysiology Assessed by Rhodamine-123 Transport and Fluorescence." *Annals of Biomedical Engineering* 35.7 (2007): 1276-285.
- Kaur, Parvinder, Michael Aschner, and Tore Syversen. "Glutathione modulation influences methyl mercury induced neurotoxicity in primary cell cultures of neurons and astrocytes." *Neurotoxicology* 27 (2006): 492-500.
- Toimela, Tarja , and Hanna Tahti. "Mitochondrial viability and apoptosis induced by aluminum, mercuric mercury and methyl mercury in cell lines of neural origin." *Archives of Toxicology* 78.10 (2004): 565-574.
- 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.
- Morris, Robert L., and Peter J. Hollenbeck. "The Regulation of Bidirectional Mitochondrial Transport Is Coordinated with Axonal Outgrowth." *Journal of Cell Science* 104 (1993): 917-27.