The effects of mercury exposure on *Gallus gallus* glial cell retraction

Allison Moreau

Neurobiology Short Report

Bio 324/Neurobiology

Wheaton College, Norton, Massachusetts, USA

April 24, 2013

Introduction:

Mercury is an environmental toxin that readily crosses the blood-brain barrier through the L-type large neutral amino acid transporter (LAT1) (Ni et al., 2011). Seafood consumption is a major environmental source for humans. Mercury toxicity in development causes neurological problems including memory and learning disabilities (Johansson et al., 2007). Many studies have looked at the effects of mercury on neurons, but not as much has been studied on its effect in glial cells, even though this is the nervous system cell type with the highest concentration of mercury (Ni, Li, Rocha, Farina, & Aschner, 2012). In general, mercury has been shown to degenerate the neuronal cytoskeleton (Eskes et al., 2002), which leads to cell retraction. Without the structural support of the cytoskeleton the cell cannot maintain its shape. The specific mechanisms for this degeneration include inhibiting tubulin polymerization (Sager et al., 1982) and stimulating microtubular fragmentation (Choi et al., 1980). In other words, mercury breaks apart the cytoskeleton and prevents it from rebuilding new tubulin.

Mercury not only damages the glial cells in this generic manner, but also causes further harm to the nervous system as a result of the loss of glial cells' protective and supportive properties. Glial cells are responsible for modulating neurotoxicity, so mercury is destroying the very cells that are meant to detoxify it (Sakamoto et al., 2008). Tied to this modulation, microglia are important in the immune response and cell repair; they secrete interleukin-4 (IL-4), an immune response protein, which triggers neuronal repair and regeneration (Ni et al., 2012). Decreased microglia levels means decreased IL-4 levels and a compromised immune system. Another general effect of mercury on glial cells is the production and secretion of lysosomal proteases out of the cell (Sakamoto et al., 2008). These proteases degrade the structure and function of proteins, so their mercury-induced secretion effectively destroys the cells it encounters.

Mercury affects not only microglia but also astrocytes. One way is mercury inhibits cysteine uptake in the

astrocytes. This prevents glutathione synthesis, an antioxidant, which leads to oxidative damage (Allen et al., 2002). Another way it disturbs astrocytes is by changing the production and reuptake of the neurotransmitter glutamate. Mercury causes the astrocytes to release excessive amounts of glutamate into the synapse. At the same time, it also prevents the astrocyte from being able to re-uptake any glutamate from the synapse to reduce its extremely high levels. Combined, this leads to neurotoxicity. Thus, there are many different mechanisms through which mercury can cause damage to the Central Nervous System.

In this study, we looked at the cytoskeletal structural effects of mercury on cell size. We tested the hypothesis that mercury exposure causes glial cell retraction. To test this, we used an in vivo primary culture of *Gallus gallus* embryonic peripheral neurons. We measured cellular retraction by comparing the change in projected surface area of chick sympathetic glial cells in a control condition with the same glial cells exposed to mercury.

Materials and Methods:

Sympathetic nerve chains and dorsal root ganglia were dissected from 10-day-old chick embryos. For dissection materials and procedure, see Morris (2013a). The primary culture used had been incubating at 37° C for 26 days before experimentation. The culture was washed with DMEM and then given fresh growth medium five days before the experiment. The day of experimentation the isolated cells were plated and a flow-chamber was made, as per Morris (2013b).

The flow chamber was observed in the Imaging Center for Undergraduate Collaboration at Wheaton College, Norton, Massachusetts using the following: an Apple iMac computer with Mac OSX 10.5.8 operating system, Nikon Eclipse E200 microscope at 40x objective magnification with phase 2 optics, Diagnostic Instruments camera model 18.2 with a 1.0x C-mount. Images were collected using Spot Software Version 4.6.09 and analyzed using ImageJ Version 1.46r software.

The flow chamber was heated to 37°C with a ceramic heater. The temperature was maintained between 37-39°C for the remainder of the experiment. The buffer was changed to a control flow of Tyrodes, using the materials and protocol for a buffer change from Morris (2013c). Images of a neuron and glial cell exposed to Tyrodes were captured using SPOT Imaging software for 4 minutes. After observing the control, the buffer was changed to a 100 nM mercury solution. The same method (Morris 2013c) used to flow Tyrodes through the chamber and remove the DMEM was used to flow mercury through as well. The same cells as the control flow were imaged for 20 minutes after exposure to mercury. Only images from the first four minutes of mercury exposure were used, so as

to match the length of control time. All images were enhanced in contrast and cropped using ImageJ software to focus on the glial cell of interest in the field of view.

The cellular behavior of retraction was quantitated in the 385 x 235 pixel region of the image with the most glial cell surface area where the cell boundary was also visible to allow for observation of retraction, as shown in Figures 1 and 2. Projected surface area of the glia in the marked region was measured in pixels using the freeform selection tool in ImageJ for 9 images. These included one initial image, four control images, each showing the cell at one minute apart, and four mercury exposure images, again each showing the cell one minute apart. The percent change in surface area from initial to final was calculated.

Results:

The projected surface area of the glial cell in the marked region decreased by 27.34% overall from the initial time the control flow began to the end of the mercury exposure. The cell maintained its size while exposed to a control Tyrodes solution, but decreased almost immediately after mercury exposure.

Figure 1

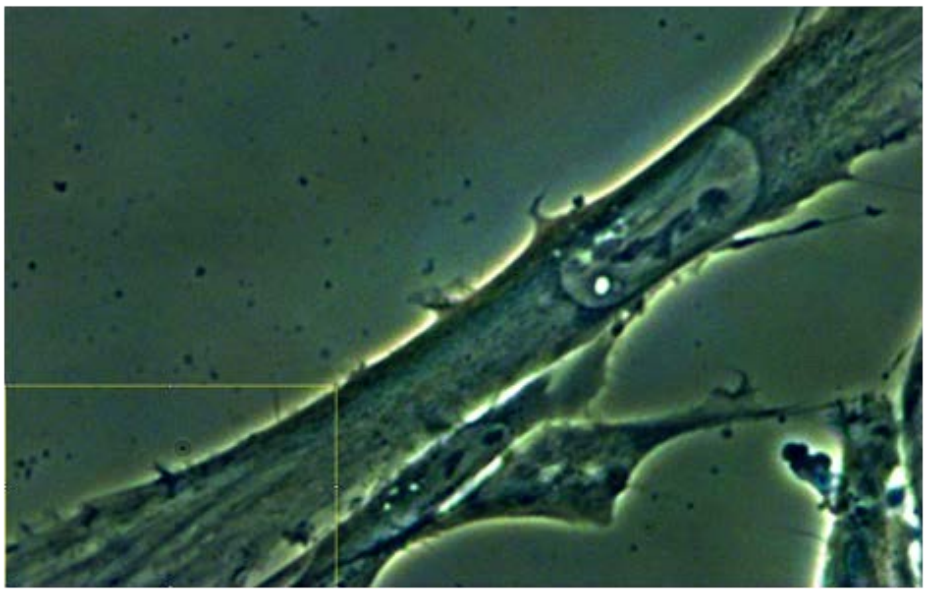

Figure 1 Legend: Glial cell control buffer flow**.** This image shows the glial cell after a control buffer of Tyrodes was applied for four minutes. Notice the amount of the region in the yellow rectangle that the cell fills before mercury treatment.

Figure 2

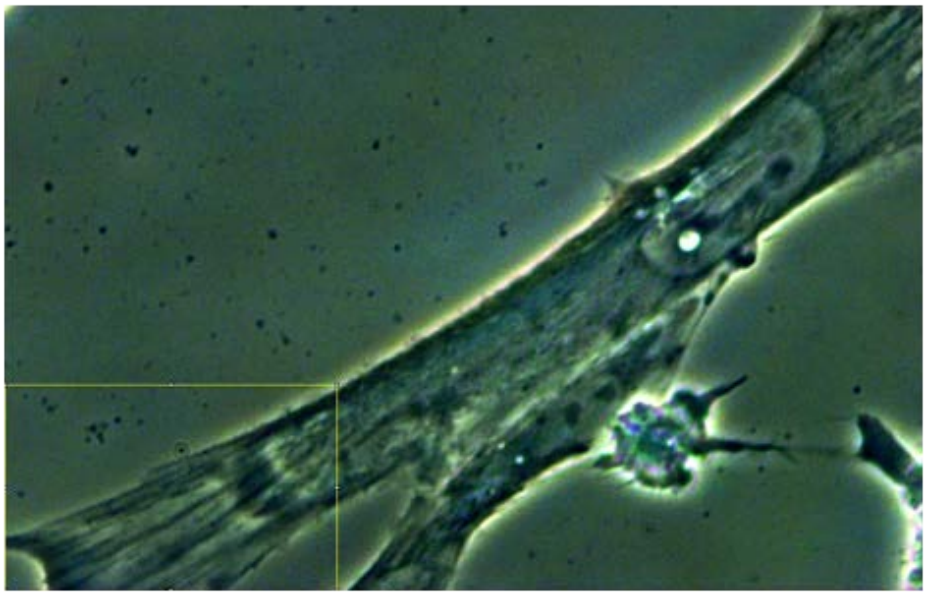

Figure 2 Legend: Glial cell exposed to mercury. This image shows the same glial cell after four minutes of mercury exposure. Notice the smaller area of the marked region now filled with the glial cell and the end of the cell coming into the field of view.

Figure 3

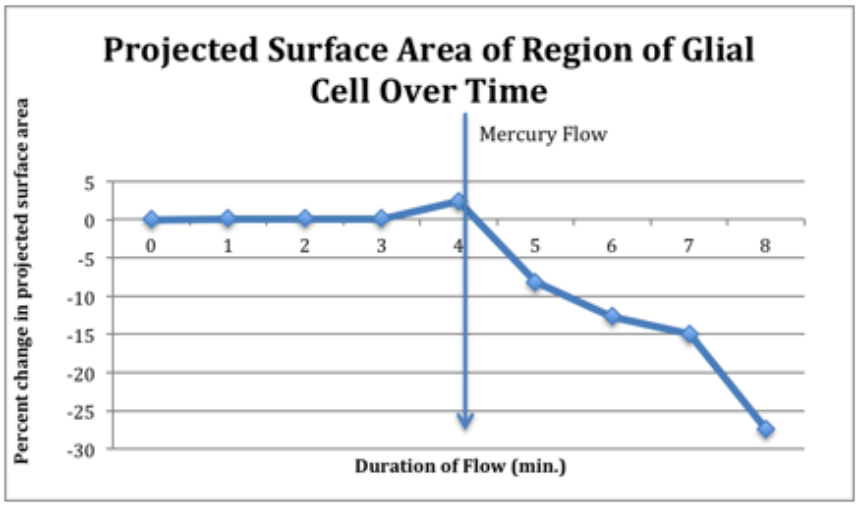

Figure 3 Legend: Change in cell shape. This graph shows the percent change in projected surface area of the glial cell in the marked region throughout the control and then mercury flow. Notice the sharp decrease in projected surface area after mercury exposure.

Discussion and Conclusion:

These findings support the hypothesis that mercury exposure will cause glial cell retraction. We can conclude that the size of this glial cell decreased after mercury flow. With large scale, statistically significant replication of these

results, the conclusion could be made that mercury consistently causes glial cell retraction. This would be due to mercury's debilitating effects on the cytoskeleton, particularly inhibiting tubulin polymerization and stimulating microtubule fragmentation (Sager et al., 1982; Choi et al., 1980). The cell will retract without the infrastructure of the cytoskeleton to maintain its shape. Therefore, a decrease in cell size, measured in this study by projected surface area, correlates to cellular retraction.

One source of error in this experiment was that the control experiment was not conducted for the same length of time as the experiment condition. The control condition was imaged for four minutes, while the mercury condition was imaged for 20 minutes. Therefore, a significant portion of the cellular retraction seen after mercury exposure could not be included in the data. The distinct cellular behavior is not as visually clear after only four minutes. If the control had been imaged for 20 minutes as well, the data would have shown a much greater amount of cellular retraction. However, the four-minute mercury time interval still showed over a 25% reduction in projected surface area of the designated region of the glial cell.

Future experiments should not only replicate this protocol to confirm the current findings, but also investigate different possible effects of mercury on glial cells, as its mechanism in neurons has already been extensively studied. A complete understanding of mercury's effect on the nervous system could lead to better preventative and treatment measures against mercury toxicity.

References Cited:

Allen, J. W., Shanker, G., Tan, K. H., & Aschner, M. (2002). The consequences of methylmercury exposure on interactive functions between astrocytes and neurons. *Neurotoxicology,* 23, 755–59.

Choi, B. H., Cho, K. H., & Lapham, L. W. (1980). Effects of methylmercury on DNA synthesis of human fetal astrocytes: A radioautographic study. *Brain Res., 202*, 238–42.

Eskes, C., Honegger, P., Juillerat-Jeanneret, L., & Monnet-Tschudi, F. (2002). Microglial reaction induced by noncytotoxic methylmercury treatment leads to neu- roprotection via interactions with astrocytes and IL-6 release. *Glia, 37*, 43–52.

Jacobson-Hardy, Leah. (2013). Personal Communication.

Johansson, C., Castoldi, A., Onishchenko, N., Manzo, L., Vahter, M., & Ceccatelli, S. (2007). Neurobehavioral and molecular changes induced by methylmercury exposure during development. *Neurotoxicity Research, 11*(3,4), 241-260.

Morris, R. L. (2013a). Neurobiology Bio324 Primary Culture of Chick Embryonic Peripheral Neurons 1: DISSECTION. Available at http://icuc.wheatoncollege.edu/bio324/2013/morris_robert/BIO324_Lab_Proc_1_Dissection.htm.

Morris, R. L. (2013b). Neurobiology 324 Primary Culture of Chick Embryonic Peripheral Neurons 2: OBSERVATION of LIVE UNLABELED CELLS. Available at http://icuc.wheatoncollege.edu/bio324/2013/morris_robert/BIO24_Lab_Proc_2_ObserveLiveUnstained.htm

Morris, R.L. (2013c). Neurobiology 324 Primary Culture of Chick Embryonic Peripheral Neurons 3: DYING and OBSERVING LIVE CELLS. Available at http://icuc.wheatoncollege.edu/bio324/2013/morris_robert/BIO324_Lab_Proc_3_StainAndObserv.htm.

Ni, M., Li, X., Yin, Z., Sidoryk-Wegrzynowicz, M., Jiang, H., Farina, M…& Aschner, M. (2011). Comparative study on the response of rat primary astrocytes and microglia to methylmercury toxicity. *Glia, 59*, 810-820.

Ni, M., Li, X., Rocha, J., Farina, M., & Aschner, M. (2012). Glia and methylmercury neurotoxicity. *Journal of Toxicology and Environmental Health, 75*, 1091-1101.

Sager, P. R., Doherty, R. A., & Rodier, P. M. (1982). Effects of methylmercury on developing mouse cerebellar cortex. *Experimental Neurology, 77*, 179–93.

Sakamoto, M., Miyamoto, K., Wu, Z., & Nakanishi, H. (2008). Possible involvement of cathepsin B released by microglia in methylmercury-induced cerebellar patholog- ical changes in the adult rat. *Neuroscience Letters, 442*, 292–96.

I have abided by the Wheaton College Honor Code in this research report.

Allison Moreau