The effects of mercury on the retraction rates of one-day old and thirty-five day old glia cells

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I. Introduction

Mercury, a common environmental pollutant found within the earth’s crust, water, and living plants and animals (Davidson, Myers, Weiss, 2004), is a toxin that severely affects the nervous system (Ni et al., 2011), particularly during fetal development and in infants by impairing their cognitive and behavioral development (Dorea & Donangelo, 2006). Once ingested by a living organism, mercury is actively transported throughout the body and across the blood brain barrier via the L-type large neutral amino acid transporter, otherwise known as LAT1 (Yin et al., 2008), where it adversely affects neurons and glia (Ni et al., 2011). The presence of healthy glia cells is imperative for providing physical support and essential nutrients (Hamilton, Hillard, Spector, & Watkins, 2007), the removal of pathogens (Teismann et al., 2003), immune responsiveness (Watkins & Maier, 2002), and maintaining optimal brain function (Ni et al., 2011), all of which are crucial to the vitality of the nervous system and overall health of an organism.

Mercury exposure adversely affects glia cells rapidly and at an expedited rate in comparison to other nervous cells. In a study conducted by Ni et al., 2011, the introduction to mercury on glia cells causes immediate oxidative stress, which is the precursor to cell death, while astrocytes respond later to the presence of mercury, thus making glia cells the first line of cellular defense against mercury introduction and therefore the first affected. Another study observed the effects of mercury (HgCl₂) and monomethylmercury chloride (McHgCl) on five to ten day old glia cells and twenty-five to thirty-five day old glia cells and discovered that HgCl₂ has a stronger effect on younger glia while McHgCl displays a higher toxic effect on mature glia (Monnet-Tschudi, Zurich, & Honegger, 1996), further demonstrating mercury’s overall toxicity in glia.

Similar studies have found that neurons exposed to mercury display a heightened retraction rate of their neurite processes and at certain doses of mercury, abnormal growth or degeneration also occur (Xu et al., 2012). Since mercury poisoning vastly impairs cellular nervous growth, it has been suggested that mercury exposure is a potential causative factor in the development of autism spectrum disorder due to its interference in microtubule function and neuronal...
migration, as seen with glia cells (Bernard et al., 2001). Prenatal mercury exposure has also been linked to reduced performance by children on tests of cognitive development, motor skills, as well as tests of behavior and attention (Axelrad, Bellinger, Ryan, Woodruff, 2007).

The purpose of this study is to test the effects of mercury on various glial cell retraction rates through the exposure of mercury on dorsal root ganglia and sympathetic nervous chains harvested from ten-day old *Gallus gallus* chick embryos, an important scientific model. It is hypothesized that the presence of mercury will cause immediate retraction of glia due to their heightened sensitivity to the toxin, as explored by Ni et al., 2011, with the younger glia demonstrating a decline in retraction rate earlier than the older glia. Since mercury hinders neuronal development, exploring the retraction rate of glia cells and how glia cells are affected differently in relation to their level of maturity in its presence may provide further evidence for mercury’s adverse affects, those of which may lead to the development of various psychiatric disorders, such as autism.

II. Materials and Methods

*Dissection:*

Ten-day old chick embryos were dissected for their dorsal root ganglia (DRG) and sympathetic nervous chains (Morris, 2013a). The materials utilized for the chick dissection can be found in Morris (2013a). Harvested DRGs and sympathetic nervous chains were placed in petri dishes for growth following Morris (2013a).

*Observation Preparation:*

The harvested nervous cells were plated on microscope slides following the flow chamber procedure outlined by Morris (2013b.) immediately before observation in the Imaging Center for Undergraduate Collaboration (ICUC) in the Mars Center for Science and Technology at Wheaton College, Norton, Massachusetts. One set of nervous cells were harvested twenty-four hours prior to observation and another set of nervous cells were harvested thirty-five days prior; both sets of nervous cells were incubated at 37°C.

*Observation:*

After plating, harvested neurons were observed in the ICUC on a Nikon EFD-3 microscope on a Mac OSX Version 10.5.8 at 40x magnification and on phase optics 100, 40. A Windmere heater was placed near the microscope and a thermometer attached directly to the microscope stage. The neurons were heated to 34°C - 40°C during experimental observations. Observations were recorded via a Sony DFW-x700 camera. The imaging software utilized was BTV version 6.0b1. Once ideal temperature conditions of 34°C - 40°C were reached, photographs were taken for fifteen minutes at fifteen-second intervals. Immediately following, 0.5mL of a 100nM solution of HgCl₂ was flowed through the chamber for one minute until the original medium was not present. Photographs of the mercury-exposed...
cells were taken for fifteen minutes in fifteen-second intervals.

**Data Collection:**

Data collection and analysis was completed through the use of the computer programs ImageJ Version 1.46r, Adobe Photoshop Version 12x64, and Microsoft Excel Version 14.2.2. Three images were utilized from the one-day old glia control images, one-day old mercury exposed glia images, thirty-five day old glia control images, and thirty-five day old mercury exposed glia images. The images utilized from each trial were time stamped at 0 minutes, 5 minutes, and 10 minutes intervals. The images were then cropped to a 300 x 300 pixel box on Adobe Photoshop and opened in ImageJ. In ImageJ, the images were stacked and an area was randomly chosen and marked by a 180 x 180 pixel box. The box was separated horizontally at 90 pixels, the top half representing section one and the bottom half representing section two.

Within each section a marker was placed halfway along the right side of the box. From this designated point a line was drawn until it reached a glial body (Figure 1). Length measurements were calculated in pixels by using the “Analyze” menu and then the “Measurement” option in ImageJ for each of the three images per environmental condition.
Length measurements were recorded for each section of the images for the three different time stamps. If a cell experienced growth, it was recorded as negative length. Retraction rates per section were calculated by the change of distance over five minutes in each section of one condition and then the two sections of one condition were averaged together. If growth occurred in one section, the data was not calculated in the average. Average retraction rates were graphed on Microsoft Excel. The retraction rates of the one-day old glial cell and the thirty-five day old glial cell in the control condition were compared in one graph and the retraction rates of the one-day old glial cell and the thirty-five day old glial cell after exposure to mercury in another.

III. Results:

Both the one-day old glial cell and the thirty-five day old glial cell displayed retraction in the control and mercury conditions. However, the one-day old glial cell in section one did demonstrate growth during the entire ten-minute interval, so data from section one were not included in the retraction rate averages.

In the control conditions, the one-day old glial cell demonstrated a faster rate of retraction through a visually observed reduction in the length of the glia as seen from Figure 2a to Figure 2b while the 35-day old glial cell displayed a decline in movement over the ten minutes as seen by some growth between the two protruding area of the glia (Figure 3a & Figure 3b). In the mercury conditions, however, the one-day old glial cell showed a slight increase followed by a decline in retraction rates while the thirty-five day old glial cell demonstrated a consistent increase in its retraction rate.
Figure 2: The first image depicts the one-day old glia cell after ten minutes of exposure to the control conditions. The arrows indicate the two glia masses that were observed during the ten minute period. The second image depicts the same glia cell after ten minutes of mercury exposure with the same observation areas highlighted.
**Figure 3:** The first image depicts the 35-day old glial cell after ten minutes of exposure to the control conditions. The arrows indicate the two glia masses that were observed during the ten minute period. The second image depicts the same glial cell after ten minutes of mercury exposure with the same observation areas highlighted.
b.)

Figure 4: This graph displays the retraction rate in pixels/minute of the one-day old glia cell in comparison to the thirty-five day old glia cell in the control conditions (Figure 4a). This graph compares the retraction rates in pixels/minute of one-day old glia cells and thirty-five day old glia cells after exposure to mercury (Figure 4b). The different time intervals were zero minutes, five minutes, and ten minutes (n = 3).

IV. Discussion and Conclusion:

This experiment supports the hypothesis that exposure to mercury causes immediate retraction within the first minute of exposure of the glia cells with the younger glial cell demonstrating a decline in retraction rates earlier than the older glial cell. Once exposed to mercury, glia cellular function decreases due to the toxic effects of the substance, which are usually caused by the immediate onset of oxidative stress (Ni et al., 2011). As such, there is an immediate retraction rate, but it is drastically reduced within five minutes in the one-day old glial cell but retraction continues at an increased rate in the thirty-five day old glial cell. It can be suggested that the immaturity and smaller size of the one-day old glial cell may cause the mercury affects to occur more rapidly, thus causing an immediate retraction quickly followed by a decline in movement as the mercury affects the cell. In contrast, the thirty-five day old glial cell displays a steady decline because its cell mass is vast and its structure complex, thus resulting in a longer amount of time needed.
for the mercury to impose its adverse effects.

A similar experiment was conducted on aggregating cell cultures of fetal rat telencephalon, which were harvested at five to fifteen days during the developmental period and between twenty-five and thirty-five days during advanced maturation; the cells were treated with mercury chloride. As mentioned earlier, the study found that younger glia cells exhibited a larger loss of enzymatic activities while the older glia cells exhibited similar but less drastic cellular loss. The older glia cells were affected more by monomethylmercuric chloride (Monnet-Tschudi et al., 1996).

Therefore, the one-day old glial cell may exhibit higher retraction rates in the control condition than the thirty-five day old cell because it is new, move more frequently, and is more effected by HgCl₂ (Monnet-Tschudi et al., 1996). The area of the cell observed may be opposite of the growth cone and thus heightened retraction in control environments would demonstrate active growth. Likewise, the thirty-five day old glial cell has already formulated an intricate, layered structure and does not need to grow and expand as drastically as the younger glial cell, which may explain the decline in retraction rate in the control condition of the older glial cell. However, more studies must be conducted comparing the effects of mercury on glia cells of differing ages in order to reach more sound conclusions.

From this data is can be concluded that mercury does indeed have toxic effects on glia cells (Ni et al., 2011; Bernard et al., 2001), especially on younger glia. If this experiment was repeated numerous times and statistically significant data was collected, it could possibly support the hypothesis that mercury ingestion early on in pregnancy may have stronger affects than mercury ingested later on and could support the theory that mercury play a role in the development of autism spectrum disorder and other learning deficiencies (Bernard et al., 2001; Axelrad et al., 2007). However, a major source of error in this experiment is that the entire glia cell is not observed in either condition, which does not allow for the determination of the growth cone direction that of which could explain some of the witnessed retractions. This experiment could be improved upon by running trials for longer periods of time and also with glia cells entirely present in the view field. Additionally, a buffer could have been utilized during the control instead of just the growth medium. Different concentrations of mercury could have also been used to determine how quickly and to what severity each concentration affects retraction rates.

As such, future experiments should be conducted over a longer time lapse to observe whether mature glia cells will eventually display a decline in retraction rates as the immature glial cell did.

V. References:


Watkins, L.R., Maier, S.F. (2002). Beyond neurons: evidence that immune and glial cells
