Decrease in glial-glial cell interactions after exposure to mercury

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Introduction

The central nervous system is made up of neurons and glial cells (Morris, 2013). For many years, it was thought that neurons were the main contributor to the functions of the nervous system and that glial cells were space fillers. However, recent studies show that glial cells are responsible for the proper development and function of the nervous system (Parker, Auld, 2006).

In an embryo, glial cells are responsible for forming a framework that permits the development of the rest of the nervous system. Glial cells also regulate neuronal survival and differentiation (Jessen, 2004). In adults, glial cells form myelin sheaths around the axons of the peripheral nervous system, which allows for faster conduction of neuronal signaling. They are also responsible for maintaining the concentrations of ions and neurotransmitters within the nervous system (Jessen, 2004). Glial cells also provide nourishment, insulate synapses, and scavenge cellular debris and dead neurons (Morris, 2013).

Mercury is known to cause damage to the central nervous system, especially during development. Some common symptoms of mercury poisoning in the human population include mental retardation, primitive reflexes, problems with coordination, dysarthria, growth disorders, and hyper salivation (Ni, 2011). These are serious disorders that can greatly impact an individual's life. In order to figure out the causes of these disorders, scientists must study the effects of mercury on different cell types. Since the central nervous system is known to be impacted by mercury, glial cells are one of the types of cells that must be studied. The neurotoxicity of mercury depends on the stage of development of the nervous system. The developing central nervous system is much more sensitive and susceptible to mercury poisoning than the adult central nervous system (Ni, 2011). This suggests that glial cells, which aid in forming the nervous system, are very vulnerable to mercury poisoning.

Glial-glial interactions play a large role within the central nervous system. One function for glial-glial cell interactions is to assist in glial migration, or the movement of glial cells (Silies, et al., 2007). There are three main types of glial cells within the central nervous system. They are astrocytes, microglia, and oligodendrocytes (Ni, 2011). The interactions between these glial cells are essential to the successful development of the central nervous system. Because of this, it is important for glial-glial cell interactions to be studied with regards to mercury poisoning.

In previous studies, it has been shown that glial cells, especially astrocytes and microglia, retain large deposits of mercury. In fact, the total accumulation of mercury within glial cells is significantly higher than the accumulation within neurons (Ni, 2011). Based on this, one can guess that glial cells retaining the mercury within the central nervous system may cause the long-term damage seen in individuals with mercury poisoning.

In this study, I tested the hypothesis that glial-glial cell interactions would be inhibited in the presence of mercury. Because glial-glial cell interaction helps with the development of the central nervous system and mercury causes damage to the central nervous system, especially within the development stages, it seems logical that glial-glial cell interactions would decrease with the addition of mercury.

In order to test this hypothesis, glial cells from 10-day-old embryonic chick embryos (*Gallus gallus*) were used. These chick glial cells were treated with a mercury-containing buffer and the differences in glial-glial cell interactions based on the number of interactions before and after mercury exposure were recorded.

Materials and Methods

For this experiment, the coverslips were cleaned and treated with poly-lysine and laminin exactly as discussed in the Primary Culture of Chick Embryonic Peripheral Neurons 1: DISSECTION Lab (Morris, 2013). The chick embryos were then dissected by R.L. Morris as described in the Primary Culture of Chick Embryonic Peripheral Neurons 1:

DISSECTION Lab. The one variation from the procedure described in this lab was that DMEM was used as a buffer rather than HBSS. R.L. Morris also carried out the procedure for dissociating the ganglia and growing them up on coverslips as described in the same lab (Morris, 2013). These dissociated cells were then placed in an incubator.

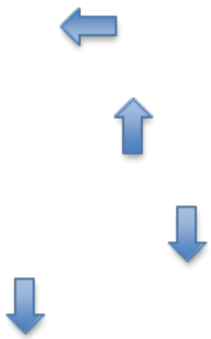
Upon removal from the incubator, the coverslip was used to create a flow chamber as described in Primary Culture of Chick Embryonic Peripheral Neurons 3: STAINING and OBSERVATION of LIVE CELLS (Morris, 2013). The flow chamber was then taken into the Imaging Center for Undergraduate Collaboration at Wheaton College in Norton, Massachusetts for observation. The cells were placed on a Nikon EFD-3 microscope, which is equipped with a Sony DFW-x700 camera and connected to an Apple iMac computer running OSX Version 10.5.8. The cells were observed using phase optics for 10 minutes in the growth medium using a heater to maintain a temperature around 37 degrees Celsius. During this time, a picture was taken every 15 seconds. Images were captured using BTV Software version 6.0b1, with the 40x objective lens and a 1.0x camera mount.

Approximately 0.5 mL of a buffer containing 100nM $HgCl_2$ was flowed through the flow chamber, as described in the section regarding Imaging Cell Behaviors After Buffer Change in the Primary Culture of Chick Embryonic Peripheral Neurons 3: STAINING and OBSERVATION of LIVE CELLS lab (Morris, 2013). However, instead of exchanging the solution for more growth medium, it was exchanged for a buffer containing $HgCl_2$. The same cells that were observed before the mercury addition were imaged again, with the same equipment used above and in the same manner as described above. The cells were observed while in the presence of mercury for 10 minutes while being heated to 37 degrees Celsius, with images being taken every 15 seconds.

Image data were opened using the ImageJ software. A section of the image that was 308x252 pixels with the left top corner at x=368 pixels, y=96 pixels was cropped out of 11 images from each trial, each one minute after the previous one. This section of the image was chosen because there were interactions between two distinct glial cells, with no interference from other cells. The width of every visible interaction between the two glial cells in this image was measured. If the interaction was over five pixels, or 7.9 micrometers, in width, it was counted as an interaction. If not, it was disregarded. Each of the images was analyzed in this manner and the results were analyzed using Microsoft Excel.

Results

The number of glial-glial cell interactions that had a width of more than 5 pixels (7.9 micrometers) decreased from the control (cells in growth medium) to the experimental (cells in a mercury-containing buffer). The average number of these interactions for the 10 minutes that the cells were in the control was 5.91, while the average number of these interactions for the 10 minutes that the cells were in mercury was 3.64. Figure 1 shows the glial cell interactions at the beginning of the analysis while the cells were in growth medium. In this image, there are a high number of glial-glial cell interactions that measure over 7.9 micrometers (5 pixels). Figure 2 shows the glial cell interactions at the end of the analysis after mercury had been added. There are visibly fewer interactions in this image than in Figure 1. Figure 3 shows the number of glial-glial cell interactions that measured over 7.9 micrometers in width in a line graph. The individual lines represent the control (cells in growth medium) and the experimental (cells in a mercury-containing buffer) over time. It is evident from this graph that the number of glial-glial interactions is lower while the cells are exposed to mercury and higher when the cells are in growth medium.



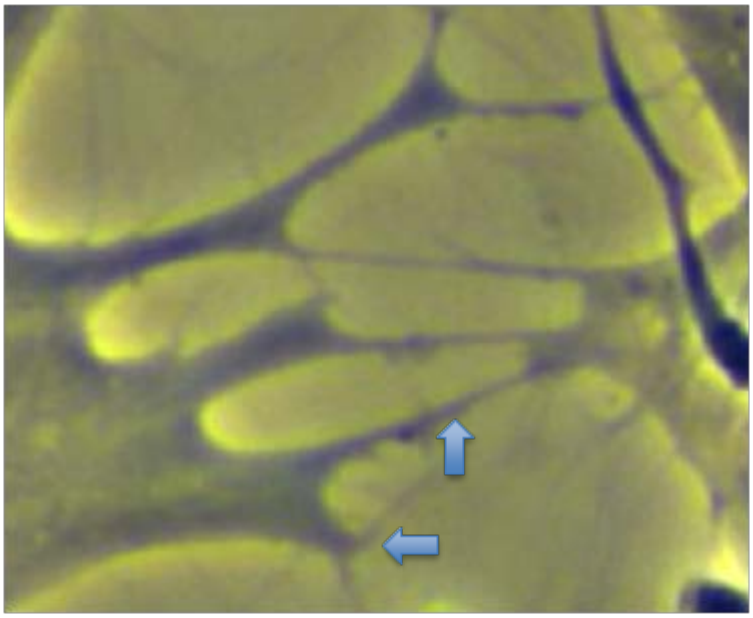


Figure 1: Glial-glial cell interactions at beginning of analysis while the cells were in growth medium. Notice the high number of visible interactions between the glial cells in this image, each of which is indicated with an arrow.

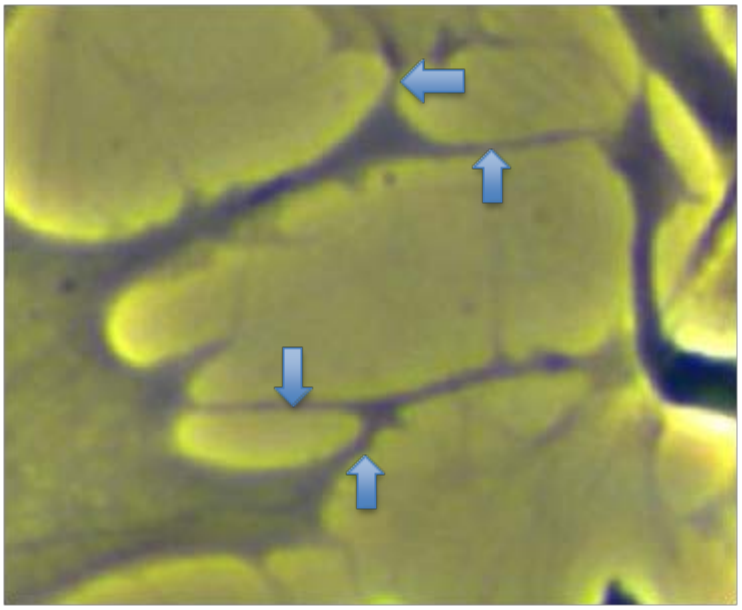


Figure 2: Glial-glial cell interaction at the end of the observation with the cells in mercury. Note the decreased number of interactions between the two glial cells, which are shown using the arrows. Further, notice the increased distance between the two glial cells.

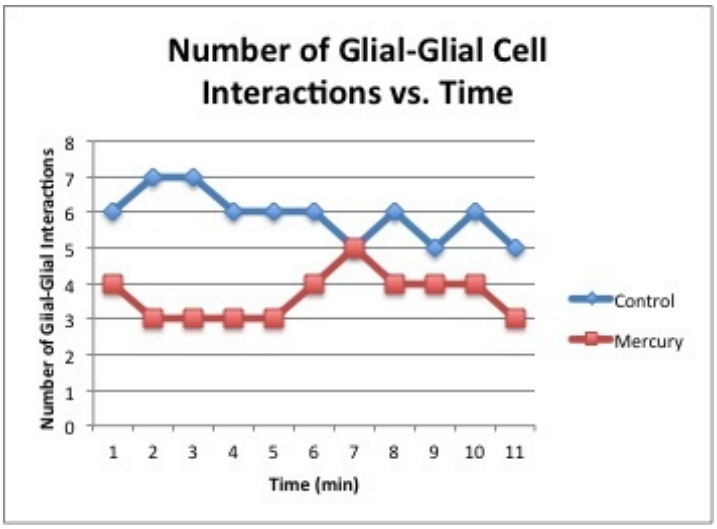


Figure 3: Number of glial-glial interactions greater than 5 pixels (7.9 micrometers) in width in growth medium and in mercury buffer over time. Note the higher number of glial-glial interactions in the control and the lower number of interactions in the cells treated with the mercury buffer.

Discussion and Conclusions

Based on this study, it seems that the number of glial-glial cell interactions decreases when mercury is added to the cells. Although the interactions decreased, they were not completely inhibited as my hypothesis suggested. Thus, my hypothesis was not supported by the data.

The data suggest that the number of glial-glial cell interactions was decreased in the presence of mercury. The analysis was done using the same portion of each of the images, which contained two glial cells interacting without further interference from other cells. While the cells were in growth medium, the average number of glial-glial interactions that measured over five pixels (7.9 micrometers) in ImageJ was 5.91. The average number of glial-glial interactions in the presence of mercury was 3.64. From this, one can argue that the mercury does decrease the number of cell-cell interactions, although it does not inhibit it.

If this experiment were run multiple times and the results were similar to those found in this trial, or if the results further differentiated between the control and experimental, it could be asserted that mercury was responsible for the decreased number of glial-glial interactions. It has been shown that mercury has a large impact on the central nervous system, causing many different disorders. Because mercury is a toxin, it decreases the ability for cells to interact with one another, which causes negative side effects (Ni, 2011). It is known that the effects of mercury are the strongest during development. Glial cells are responsible for forming the framework that allows for the development of the rest of the nervous system (Jessen, 2004). Thus, it is likely that glial-glial cell interactions would decrease when exposed to mercury.

There are three things that should be changed if this experiment is run again. First, instead of flowing the mercury through right after the growth medium, DMEM or another buffer should be flowed through first. Mercury rapidly attaches to proteins, which we know are prevalent in the growth medium (Morris, 2013). Thus, flowing a buffer through the flow chamber first would decrease the number of proteins outside of the cell for the mercury to attach to, allowing the mercury to have the largest impact on the cells. Second, the control for the experiment should be a Tyrode's salt solution rather than growth medium. This is much more comparable to the mercury-containing buffer that was used, so the results from this experiment would allow for much more significant conclusions. Finally, this experiment should be run for a longer time in order to determine if the longer exposure to mercury would have a greater impact on the glial-glial cell interactions. Studies are often done to determine how chemicals or other substances will impact humans in order to benefit the human race. In order to determine the toxicity of mercury with regards to the nervous system, one would need to test whether longer exposure causes a greater impact on glial cell interactions. If there is a positive correlation between the amount of time the cells are exposed to mercury and the relative damage to the cells, it is likely that a scientist would be able to better determine the amount of exposure that cells can undergo before it is extremely harmful. This could then potentially be used to improve the health and life span of many individuals.

There are three experiments that would help to determine if the mercury is actually the cause of the decreased

number of glial-glial interactions. First, changing the concentration of mercury would show whether larger doses of mercury had a greater impact on the glial-glial cell interactions. This would assist in the determination of how much mercury exposure can be endured, allowing scientists to better estimate the amount of mercury that individuals can be exposed to without causing damage to the central nervous system. Another possible experiment would be to increase the duration of mercury exposure and see if this has a larger impact on the number of glial-glial interactions. This may create a larger difference between the control and the experimental trials, allowing for a more conclusive result. This would also help to determine the amount of mercury exposure that causes significant damage to the nervous system. The final experiment would be to make the flow chamber using a coverslip that has cells that had been incubated for a longer period of time. This would allow the glial-glial cell interactions to be stronger because they are more developed. The experiment would allow the researcher to see the impact of mercury on more developed cell interactions and determine whether this impact is as strong as it is in the cells that were studied. This could then be compared to cells within the human body. When the nervous system is developing, the cells are very similar to those that have been in the incubator for shorter periods of time. However, as an individual matures, the nervous system develops further and gets stronger. Determining whether cells that had been incubated longer experienced as great of an impact would be comparable to determining the intensity of the impact of mercury on the adult central nervous system.

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