

Effects of mercuric chloride on mitochondrial velocity in neurons and glial cells of chick dorsal root ganglia

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Introduction:

Mitochondria are organelles that provide energy to cells through oxidative phosphorylation (Kann & Kovács, 2006). They require a large amount of oxygen; the majority of the oxygen that we breathe in (about 98%) is consumed by our mitochondria. Mitochondria are responsible for providing energy to most cellular processes (Duchen, 2004). This organelle is responsible for much of the metabolic rate of the cell. In the central nervous system (CNS), the metabolic rate is extremely high. This is due to the neuron's need for a high amount of ATP (adenosine triphosphate) in order to maintain ion gradients across the cell membranes. Mitochondria are also essential to the homeostasis of calcium ions (Ca^{2+}) inside the cell (Kann & Kovács, 2006).

Mitochondria are found in the cell body, but in cells such as neurons, they are required to travel away from the cell body. Since neurons are metabolically active cells, the functioning of mitochondria is important to the life of the neuron. In neurons, the mitochondria are recruited to subcellular locations for the growth of axons and dendrites. A decrease in movement of the mitochondria down the axons of neurons can cause a lack of cellular growth (Chen & Chan, 2009). Cytochrome C (Cyt c) is an apoptosis-inducing protein that can be found in the space between the inner and outer membranes of the mitochondria. When a stimulus signals apoptosis in a cell, Cyt c is released from the mitochondria into the cytosol and cell death proteases are activated (Kondoh, Ogasawara, Araragi, Higashimoto & Sato, 2001). Research has shown that the introduction of to cells to releases Cyt c, which triggers apoptosis through the activation of caspase-3 and -9 (Araragi et al., 2003).

There is much interest in the effects that mercury has on the CNS, and specifically the brain, because mercury is

closely linked to autism. Mercury can be transported across the blood-brain barrier, making it easy for it to enter the brain and damage neurons and glia. It can also cross the placenta and accumulate in the blood and brain of a fetus. It is believed that this transfer into the fetal CNS is one cause for autism (Counter & Buchanan, 2004). In the past, mercuric chloride (HgCl_2) was used in antiseptics and in teething powders until it was discovered that it caused acrodynia when ingested and death when rubbed on the umbilical chord (“Mercury & Autism”, 2012).

The embryos from the *Gallus gallus* (chicken) are beneficial to science because they are easy to dissect and have been used for a long time, so much is known about the subject. They are also useful because they have a short development time; the entire development to hatching period only lasts 21 days. However, in this short period of time, they are able to grow to a size that allows us to see aspects of development and structures easily under a microscope (Vergara & Canto-Soler, 2012). After just 10 days of incubation the entire spinal column, wings, legs, eyes and beak are very visible.

For this experiment I tested the hypothesis that the introduction of HgCl_2 to neural and glial cells would slow the movement of mitochondria. Because HgCl_2 can lead to cell death, and mitochondrial movement is a way to measure the growth activity of neurons, I believed that there would be faster movement of the mitochondria in the control cells than in the cells that were introduced to HgCl_2 . I also believed that the cells that were introduced to 100nM of HgCl_2 would show slower mitochondrial movement than the cells that were introduced to 10nM of HgCl_2 . This hypothesis was tested by comparing control cells (incubated in growth medium) to cells that were incubated in 10nM HgCl_2 and 100nM HgCl_2 .

Materials & Methods:

Primary Culture: Coverslip cleaning and dissection

In order to carry out this experiment, cells were obtained from ten-day old chick embryos. The methods and materials used for this dissection can be found in Morris (2013a). Where HBSS (Hanks Balanced Salt Solution) is used, we used DMEM (Dulbecco’s Modified Eagle Medium) instead.

Introduction of Mercuric chloride to cells in dishes

Cells were left in the incubator at 37°C and removed from the incubator before HgCl_2 was added. Growth medium was removed from the dish and placed into the lid. Cells were washed with DMEM for 30 seconds. After removal of the DMEM, the Tyrodes and HgCl_2 were added to the cells. The control dish received growth medium, and

the experimental dishes (10nM HgCl₂ and 100nM HgCl₂) received a dilution of 1nM HgCl₂ in Tyrodes solution with additional Tyrodes solution. Once the mercury was introduced to the cells, they were incubated for 30 minutes at 37° Celsius. After incubation, cells were returned to the growth medium.

Observation of Live Cells: Chip chamber formation and ICUC

A chip chamber was created to observe the cells under a microscope (Morris, 2013b). Data was gathered at the Imaging Center for Undergraduate Collaboration at Wheaton College, Norton Massachusetts. Images were captured using a Sony digital interface (DFW-X700) camera on the Nikon Eclipse E200 light microscope at 100x magnification. Our condenser was set to Phase 2. We used a Mac OSX Version 10.5.8 computer named 'Cancer'. A heater was placed near our slides and the temperature was monitored to maintain a temperature between 34°- 39° Celsius. As the slides were heated we used the BTV version 6.0b1 imaging software to take pictures of our samples every 3 seconds for 2 minutes.

Results:

A large group of neurons were seen in the control slide. Figure 1 shows the groups of neurons that were observed. Mitochondrial movement and blebbing were observed. Mitochondrial velocity was measured by dividing the total distance moved over the amount of time it was measured. The mitochondrion measured in the control slide moved a total distance of 113.7mm in 2 minutes, giving it a velocity of 0.9481mm/s. Glial cells were observed in our slide that was introduced to 10nM of HgCl₂. Figure 2 shows the glial cell that we focused on. Movement was observed throughout the cell. The mitochondrion that was measure moved a total distance of 94.66mm in 2 minutes, giving it a velocity of 0.7888mm/s. The slide with the high concentration (100nM) of HgCl₂ also contained glial cells. Figure 3 shows the cells that we focused on. Similar movement was observed in both experimental groups of cells. The mitochondrion measured in this cell moved a total distance of 84.96mm in 2 minutes, which gave it a velocity of 0.7080.

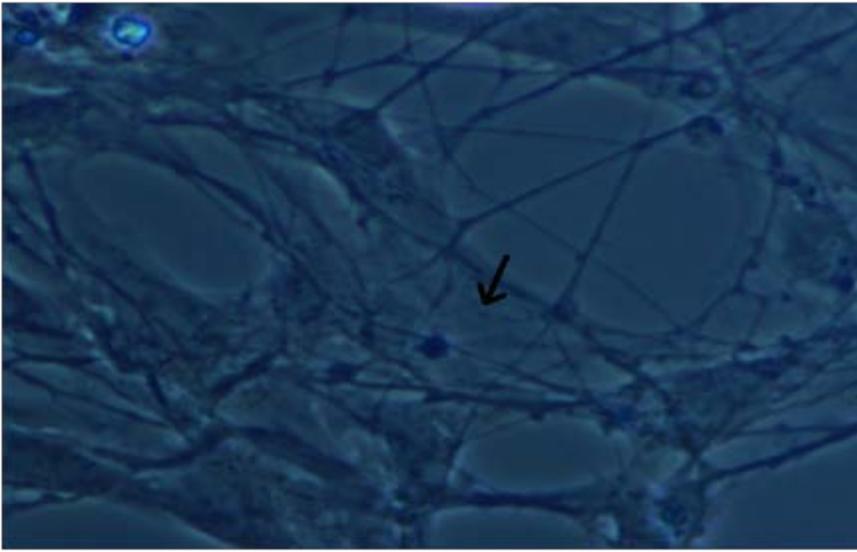


Figure 1: Cluster of neurons in control slide at start time. Arrow indicates the mitochondrion that was measured. This mitochondrion was chosen because it is clear to see and is away from overlapping axonal branching. Figure created in collaboration with Jordan Muse.

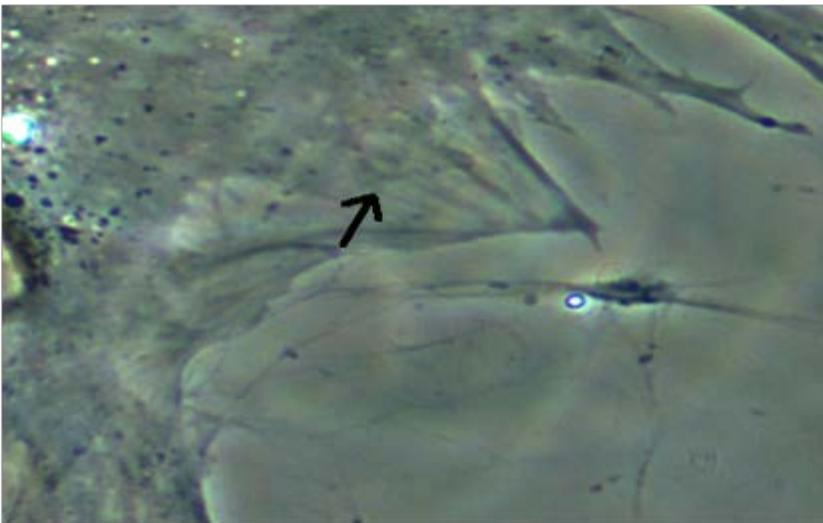


Figure 2: Glial cell introduced to 10nM HgCl₂ at start time. Arrow indicates the mitochondrion that was measured. This mitochondrion was chosen because the movement was clear. Figure created in collaboration with Jordan Muse.

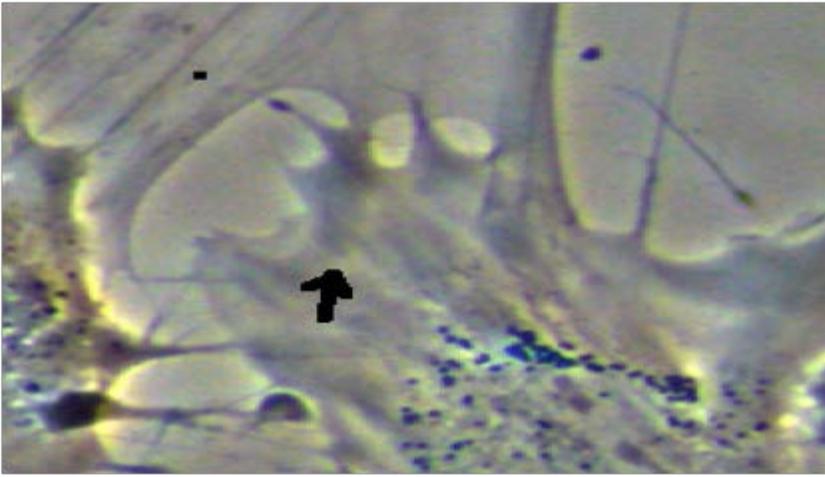


Figure 3: Glial cell introduced to 100nM HgCl₂ at start time. Arrow indicates the mitochondrion that was measured. This mitochondrion was chosen because the movement was clear. Figure created in collaboration with Jordan Muse.

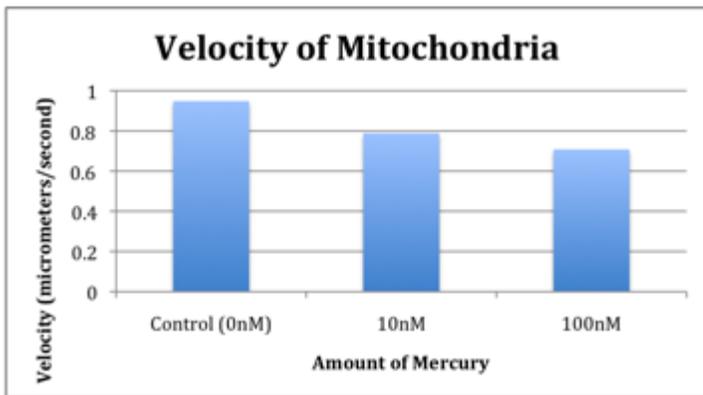


Figure 4: Velocity of the measured mitochondrion in each condition. Data are derived from measuring one mitochondrion for two minutes. Graph was produced using Microsoft Excel version 12.3.5.

Discussion and Conclusions:

The data gathered from this experiment support the hypothesis that the cells that were introduced to HgCl₂ would have slower movement than the control cells. The control cells had the highest velocity and the 100nM HgCl₂ cells had the slowest velocity. Since the 100nM of HgCl₂ had more of an effect on the cells than the 10nM of HgCl₂ did, we could say that an increase of mercury has more of an affect. This would be something that could be tested more accurately if more cells were tested. Since issues arose with growing some of our cells, it could have been that the cells that were used for our control and 10nM concentration were initially more healthy and thriving than the cells placed in the 100nM concentration because these cells were an older culture. Movement of the cells should have been measured before they were introduced to the HgCl₂. However, a chip chamber was created, and the cells were enclosed in a slide,

making it impossible to view the cells under both conditions using this procedure. If this experiment is repeated, a flow chamber should be used instead of the chip chamber so that cells can be observed before, during and after the introduction of mercury to the cells.

If this experiment were repeated many times, the same results were found, and it was tested that the results were statistically significant, we could say in more confidence that mercury has an adverse effect on mitochondria in neurons and glial cells. If these results were reliable and the trends seen in the cells were convincing, I would hypothesize that the mercury was leading to cell death because of the mitochondrial vitality to the cells. Without mitochondria, the cells would have no energy and since HgCl_2 has a damaging effect on the mitochondria, the introduction of it to the cells would cause the cell to die (Kann & Kovács, 2006).

Results could have been improved or changed by looking at the same type of cells for all three conditions of our cells; control cells were neurons, but experimental cells were glia. Data was limited since a second set of cells was unable to be harvested. With another set of data, better and more accurate results could have been possible. The slower velocity of the mitochondria in the experimental cells may have resulted from a release of Cyt c, triggering apoptosis. More research should be conducted to further understand how mercury affects the CNS. The link that mercury has to autism provides an important reason to carry out this type of research.

References:

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