Persistence of mitochondrial movement in the presence of mercuric chloride

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Introduction:

Mitochondria are essential organelles for aerobic cells, such as neurons, due to the fact that they are the main contributor of energy (ATP) in cells and in the brain (Hollenbeck & Saxton, 2005). In this study, it is hypothesized that the persistence of mitochondria movement will be reduced in the presence of mercuric chloride ($HgCl_2$). Mercury has been seen to cause neurotoxic effects on the brain due to the fact that it may lead to apoptosis of neurons (Mutter et. al, 2005). Mercury recently has been thought to contribute to some neurological disorders such as Autism and cerebral palsy and is currently being aggressively researched in order to gain more knowledge in order to prevent its toxic effects on the brain (Holmes et.al, 2003). It would be interesting to observe whether mercuric chloride has any effect on the mitochondria of neurons and glial cells. It is important to understand what movements mitochondria undergo, which proteins contribute to their movement, which proteins act as docking proteins, and what effect mercuric chloride has on these movements.

It is known that the pattern of neuronal activity within the brain is continuously changing which tells us that the mitochondria must be easily moved in order to match these changes (MacAskill & Kittler, 2009). Mitochondria are transported to regions of high metabolic demand, such as synapses, growth cones, and nodes of Ranvier. In previous studies, it is observed that 75% of mitochondria located in a neuron were persistently stationary whereas only 25% of mitochondria were observed to be mobile (Hollenbeck, 1996). These studies have defined the movements of mitochondria to be both saltatory and bidirectional. Studies have defined anterograde transport of mitochondria a result of motor proteins called kinesins, which drives the movement towards the plus-ends of microtubules (Hollenbeck & Saxton, 2005). The two proteins of the kinesin family that have been found to play a major role in this anterograde movement include kinesin-1 (KIF5B) and kinesin-3 (KIF1B) (Boldogh & Pon, 2007). These studies had also observed that inhibition of dynein altered mitochondrial distribution throughout the axon and therefore it is believed that members of the cytoplasmic dynein family controls the retrograde movement toward the minus-ends of the microtubules. (Boldogh& Pon, 2007).

The fact that a majority of mitochondria are stationary at any given moment indicates that there is likely to be some sort of docking protein that is inhibiting their motility. In many studies, the actin cytoskeleton is favored as the docking substrate due to the fact that disruption of actin filaments results in an increase in the motility of mitochondria (Hollenbeck &Saxton, 2005). Other studies have given evidence to the fact that neurofilaments could also play a key role in anchoring mitochondria with higher membrane potentials (MacAskill &Kittler, 2009). A good possibility may be that motor and docking proteins share the same system of regulation so when motor proteins are inactivated, the docking proteins are activated and vice versa (Hollenbeck, 1996).

In this study, mitochondria of chick neurons and glial cells were observed in three different solutions, the first being the control with no mercuric chloride, the second containing 10 nm of mercuric chloride and the third containing 100 nm of mercuric chloride. Phase microscopy was used to observe time spent still and time spent moving over a 2 minute time frame.

Materials and Methods:

Materials

All tissue culture media and supplements were obtained from our Lab at Wheaton College in Norton, Massachusetts. All of our materials and tissue cultures were provided by our Professor, Robert L. Morris.

Cell Culture

Sympathetic chain ganglia and dorsal root ganglia (DRG's) were dissected from 10 day old chicken embryos. For information on dissection materials and procedure, see (Robert L. Morris, 2013). The following week, the dishes of cells were retrieved from the incubator and the growth medium was removed and placed in the lids. DMEM was then added to wash out the remaining growth medium of each of the three sets of cells. Once the DMEM was removed, the control solution (tyrodes salt solution only) was added to the first set of cells. The first experimental solution made from 9nm of tyrodes solution and 1nm of $HgCl_2$ solution was added to the second set of cells, creating the experimental solution of 10nm $HgCl_2$. The 100nm $HgCl_2$ solution was created using 9nm of $HgCl_2$ and 1nm of tyrodes, which was then added to the third set of cells. The three dishes were then placed back into the incubator for 30 minutes at 37°C.

Observation of live unlabeled cells

Three separate chip observation chambers were created using the materials and procedure provided by Morris (Robert L. Morris, 2013). The observation chambers were observed in the Imaging Center for Undergraduate Collaboration (ICUC) at Wheaton College, Norton Massachusetts. The Apple I-Mac computer labeled "Cancer" with Mac OS X operating system, the Sony Digital Interface (DFW-X700) camera with a 1.0 C-mount, and Nikon eclipse E200 microscope with phase optics were used throughout the entire data collection. The microscope was aligned for Koehler illumination prior to observing any of the cells. A ceramic heater and an electronic thermometer were used to keep the cells between 37°C - 39°C. The cells were then imaged every 3 seconds for a total time of 2 minutes on 100x magnification using BTV imaging software, version 6.0b1. The images were then formed into a slideshow and mitochondria movements were observed.

Measurements of mitochondrial movements

Mitochondria were identified by their oval/ kidney bean shape. They were often more abundant towards the edge of the neuron and glial cells. One mitochondrion's persistence (time spent moving vs. time spent still) was measured in each sample, based upon the ability to determine whether the mitochondrion was moving or still throughout the entire two minutes. The mitochondria observed are shown in Figures 2, 3 and 4. The 40 different images taken of each mitochondrion were used to observe the time the mitochondria spent moving vs. the time the mitochondria spent still. The persistence ratio (time spent moving / time spent still) was then calculated for each mitochondrion.

Results:

Mitochondrial movement was observed in the control cells and both experimental cells (10nm and 100nm). In the control solution, the mitochondrion spent 66 seconds moving and 54 seconds not moving resulting in a persistence ratio of 1.22. In the 10nm solution, the mitochondrion spent 51 seconds moving and 69 seconds not moving resulting in a persistence ratio of .739. In the 100nm solution, the mitochondrion spent 47 seconds moving and 73 seconds not moving resulting in a persistence ratio of .644. Mitochondria were moving in both anterograde and retrograde directions in all three of the solutions. Less mitochondrial movement was observed as the amount of mercuric chloride in solution increased.

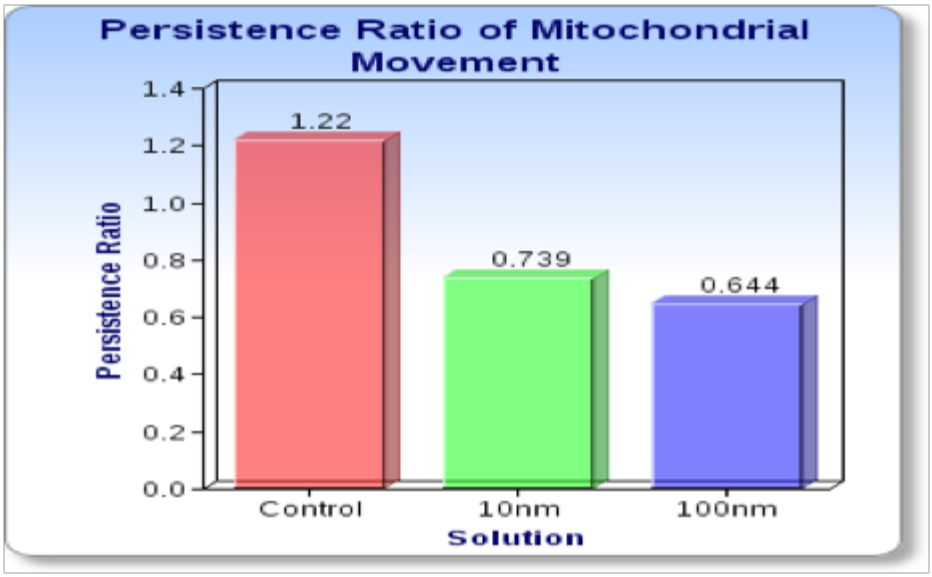

Figure 1: Graph of the persistence ratio of mitochondrial movement in three separate solutions. This graph displays the persistence ratio of mitochondrial movement during two minutes. The mitochondrion that was in the mercuric chloride free solution shows to have the largest persistence ratio. As the amount of mercuric chloride in the solution increases, the persistence ratio decreases.

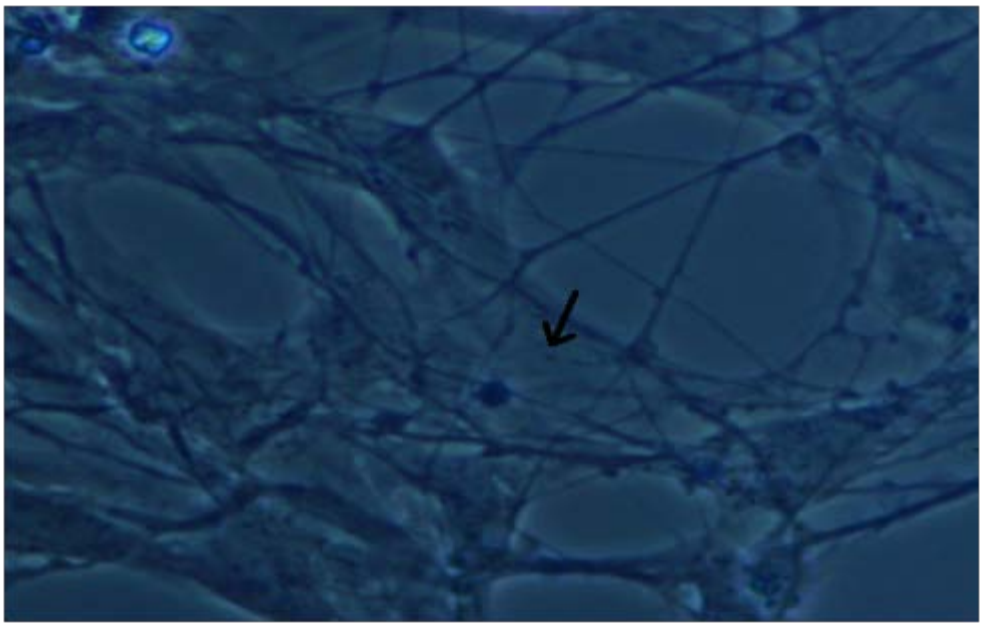

Figure 2: This figure shows a neuron that was submerged in the control solution for 30 minutes. The arrow is pointing to the mitochondrion that was observed and analyzed during this experiment. (Monahan, 2013).

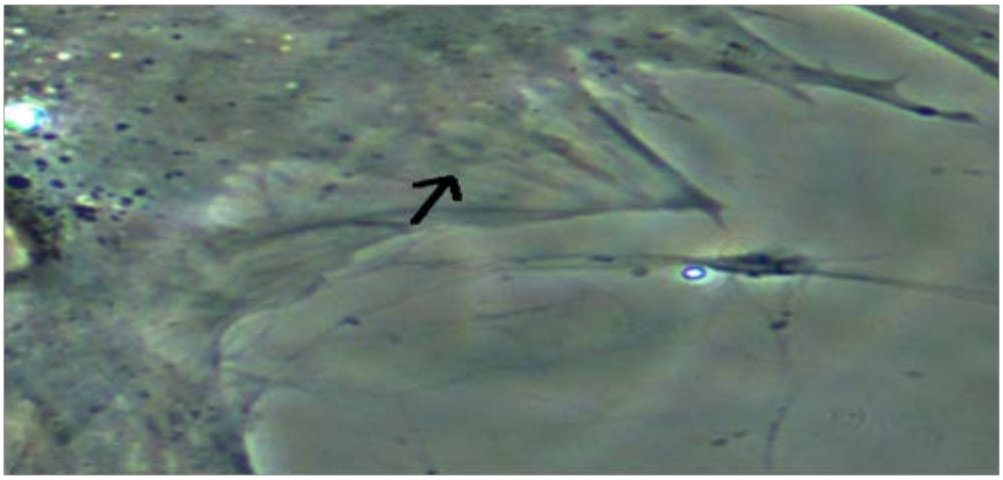

Figure 3: This figure shows a glial cell that was submerged in the 10nm HgCl2 solution for 30 minutes. The arrow is pointing to the mitochondrion that was observed and analyzed during this experiment. (Monahan, 2013).

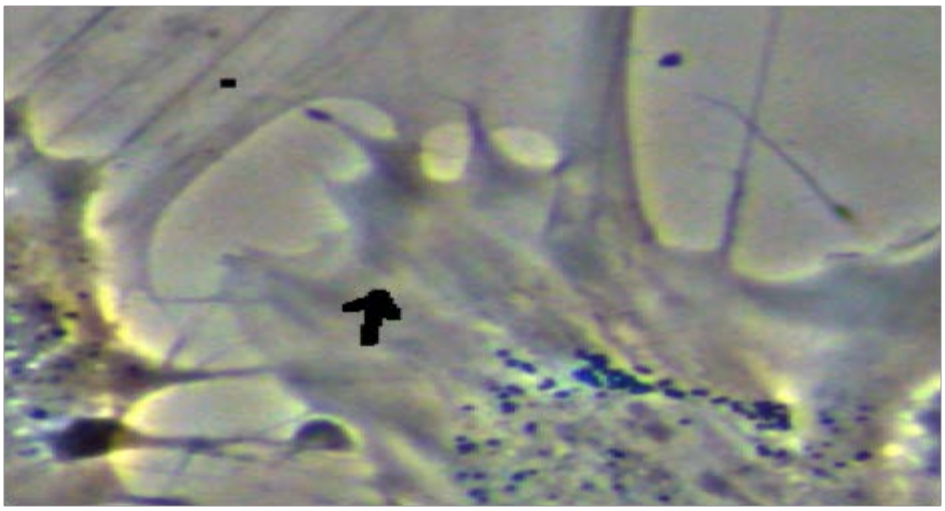

Figure 4: This figure shows a glial cell that was submerged in the 100 nm solution for 30 minutes. The arrow is pointing to the mitochondrion that was observed and analyzed during this experiment. (Monahan, 2013).

Discussion:

The data that was collected supports the hypothesis that persistence of mitochondrial movement will be reduced in the presence of mercuric chloride ($HgCl_2$). As seen in Figure 1, the persistence ratio of mitochondrial movement decreases as the presence of $HgCl_2$ increases. From these results, several different conclusions could be considered. First, one may conclude that the mercuric chloride is negatively affecting either the Kinesin-1 (KIF5B) or the Kinesin-3 (KIF1B) proteins resulting in incorrect functioning of these proteins. This may be concluded due to the idea that these kinesin-family proteins are involved in anterograde movement of the mitochondria and if they are not functioning properly than the anterograde movement will slow down or stop completely (Boldogh &Pon, 2007). Second, one can also make an assumption that the proteins of the cytoplasmic dynein family may also be negatively affected by the presence of mercuric chloride because it is thought that these proteins are involved in retrograde movement and if they are not functioning properly, they will also slow down or stop completely (Hollenbeck, 1996). However, it has also been noted that the dynein-mediated transport also requires correct kinesin function, therefore a reduction or stop in retrograde movement may also be due to the kinesin family proteins not functioning properly as well (MacAskill &Kittler, 2009). The data could also be a result of the docking proteins not working properly. Studies have concluded that the actin cytoskeleton is favored as the docking substrate due to the fact that disruption of actin filaments results in an increase in the motility of mitochondria (Hollenbeck & Saxton, 2005). With this understanding, the data suggests that it is a possibility that mercuric chloride could decrease the amount of disruption of actin filaments in the cytoskeleton resulting in a decrease in the motility of mitochondria.

In this experiment, only one set of data for each solution was able to be quantified; therefore the conclusions considered are only assumptions. Although the results obtained supported the hypothesis, retrieving data from three separate samples of each solution would strengthen this experiment. Determining how much time spent undergoing anterograde movement vs. retrograde movements to determine the net movement would also strengthen the experiment because it would give one a better understanding of which proteins were actually being affected by the mercuric chloride.

This is an important area of study because there has been a lot of research on the neurotoxic effects of mercury on the brain and its recent link to Autism. It is understood that mitochondria may play a role in apoptosis of neurons (MacAskill & Kittler, 2009) and there have been studies conducted on mercury exposure in the brain that suggest that mercury exposure could initiate apoptosis of neurons in the brain (Mutter et. al, 2005), this could be a topic of interest to other researchers. Other experiments could be created to gain a better understanding of what is happening to certain organelles such as mitochondria in the brain when they are exposed to different types of mercury. This would help researchers gain a better understanding of which types of mercury are most harmful to the brain and which organelles or regions of the brain each type of mercury is affecting. This would also allow steps to be taken to prevent the neurotoxic effects from occurring.

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