The Effect of Mercury on Direction of Mitochondrial Movement in Embryonic *Gallus Gallus* Neurons.

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Introduction:

Mitochondrial movement within neurons has been shown to be important for nerve cell growth due to the concentration in areas like the growth cones leading to higher levels of ATP production necessary for activities like cell growth (Morris and Hollenbeck, 1993). Mitochondrial movements in neuron cells are highly dependent on microtubules in the cell (Morris and Hollenbeck, 1995). Bidirectional movement of mitochondria is seen in healthy neurons and the mitochondria tend to have higher concentrations of mitochondria move out towards growth cones to aid in energy production needed for cell growth (Morris, et al. 1993). According to Leong, et al. (2001), mercury has been shown to effect microtubule metabolism by inhibiting β-tubulin from binding GTP thus inhibiting polymerization of microtubules. This inhibition of polymerization leads to a lack of microtubule growth in the axons. Hollebeck and Saxton (2005) discuss how long fast mitochondrial movements require microtubules and the organization of the plus ends of microtubules towards the nerve terminal show that the kinesin superfamily motor proteins are frequently involved in antrograde movement of mitochondria along microtubules.

In this study we have hypothesized that mercury exposure will cause an increase in retrograde and decrease of anterograde movement of mitochondria in axons, as opposed to control axons which will show more anterograde movement. To test this hypothesis, we will be performing an experiment comparing quantified mitochondrial movement in a control sample unexposed to mercury to a mercury exposed sample. In this study we took two samples of embryonic *Gallus gallus* sympathetic neurons and exposed one of them to mercury. We then measured and compared the magnitude and direction of mitochondrial movement in each of the samples over a short period of time.

Materials and Methods:

All of the preparation and imaging for this experiment was done with lab partner Seeman (2011).

Materials

The materials used for slide preparation included: forceps, HBSS and DMEM, Rhotamine 123, coverslips, slide, coverslip chips, kimwipes, pipettes, mercury working solution, aluminum foil, petri dishes, warmed valap, and paint brushes For the imaging the microscope Capricorn in the ICUC was used along with a Mac computer running MAC-OS and spot imaging software version 4.6was used along with a spot insight Camera that had a 1x lens attached. For the data collection, imagej software was used along with photoshop for the final photos placed in this report. For quantification of the data in the table and graphs, Microsoft Excel was used.

Slide preparation

The *Gallus gallus* samples were prepared using instructions in Morris, 2011a and b. The cover slips were then stained and prepared for imaging using the protocol for vital-stain fluorescence microscopy described in Morris, 2011c. There were some adjustments made from these protocols for our experiment. The Rhodamine 123 was at a concentration of 50 µg per milliliter and exposed for 10 min as explained in Morris (2011c). After completing through step 20 in Morris, 2011c for the mercury exposed sample, 2 pipettes full of the 19mL of 100nM mercuric chloride working solution was added. The mercury was exposed to the neurons for 15 minutes and then the mercury was removed from the dish and we continued as described in step 21 in Morris, 2011c.

Imaging

For the image collection, we followed the instructions described in Morris, 2011c “Observe cells on fluorescent microscope using epi-fluorescent illumination.” After collecting a phase image, we changed the exposure time down to .5 seconds and took 2 fluorescent images of 1 area 5 seconds apart for each sample. These images were saved and later used in the ImageJ program for quantification.

Quantification

Using the ImageJ program we were able to measure the distance from a fixed point near the start of the growth cone out to mitochondria of interest in both the before and after pictures. We then found the difference between the two distances to find how far the mitochondria traveled in the 5s. This was then repeated for 20 mitochondria in the control

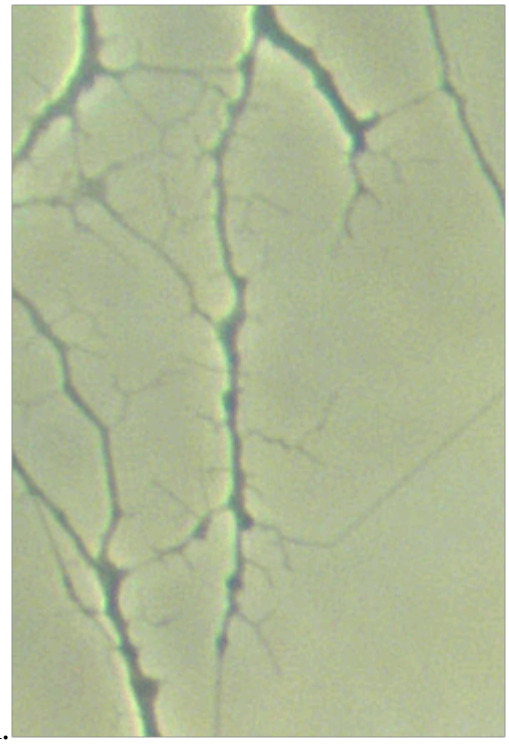
sample and 21 mitochondria in the mercury-exposed sample.

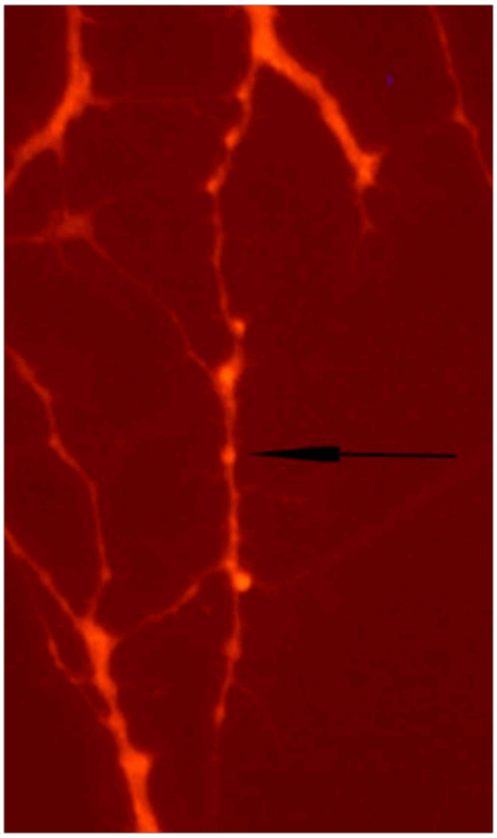
The distance each mitochondrion moved for each sample was averaged to find the total net movement after 5 seconds for both the control and the mercury-exposed samples. The distances were then separated into the mitochondrion that moved antrograde in the sample and the ones that moved retrograde in the sample. These groups were then added and divided by the number of mitochondrion in the group to find the average pixels transversed antrograde and the average pixels transverse retrograde for each sample. (Figure 1) The mitochondria were also broken up into groups based on their magnitude and direction of movement seen after 5 seconds and then the percentage of each group was found for the total mitochondria that we measured in each sample. (Figure 2)

Results:

The data indicates a net total mitochondrial movement antrograde in the control sample, and a net total mitochondrial movement retrograde in the mercury-exposed sample. When looking at the average movement in either direction for each sample, there was equal distance moved in each direction by the mitochondria though there were more mitochondria that moved antrograde. In the mercury-exposed sample there was more movement made by the retrograde mitochondria than the antrograde and more mitochondria moving retrograde. (figure 2)

When looking at the percentages in figure 3 A. and B., there was highest percentage, 65% of movement in the control between 0 and 1.99 pixels antrograde, while in the mercury exposed there is the highest percentage, 38% of the movement is between -0.01 and -2 retrograde closely followed by 28% of the movement that is between 0 and 1.99 antrogade and 24% of the movement that is between -2.01 and 4.

A. 

B. 

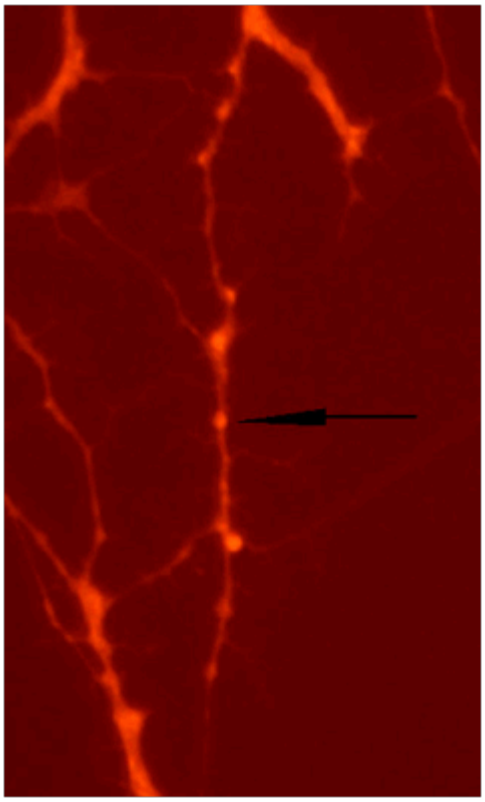
C. 

Figure 1: This figure uses 3 photos to illustrate how data was collected via imaging done in the experiment. A. This picture is the phase image of our mercury-exposed sample that was used for fluorescent imaging to track mitochondrial movement. The phase image makes it possible to see cell structures for identification of direction of movement. B. This photo is of the same area photographed under phase now in fluorescent imaging. The arrow in the center points to one of the mitochondrion that was used for measurements shown in the following figure and table. C. This photo is the same area photographed again under fluorescent imaging 5 seconds after photo B. This photo also contains an arrow pointing to the same mitochondrion in photo B but after it was allowed to move for 5 second.

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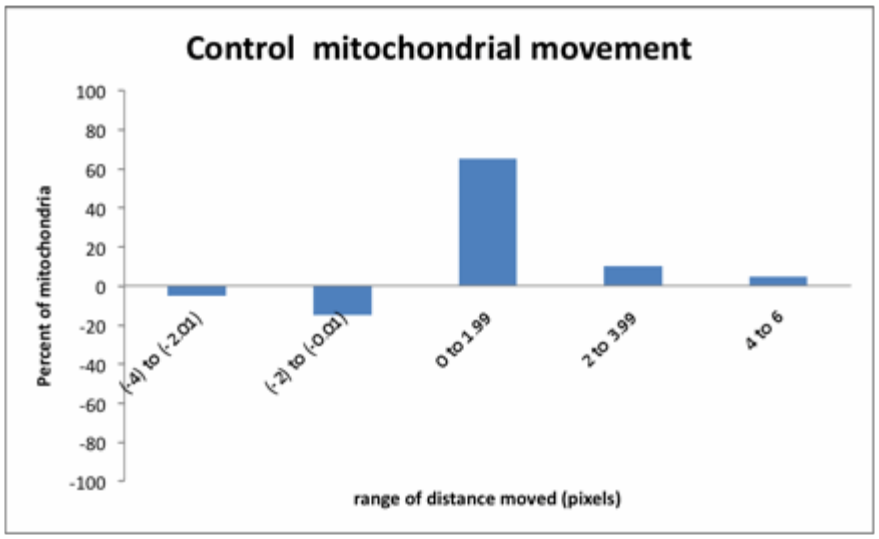
	Average Pixels Transversed Antrograde	Average Pixels Transversed Retrograde	Total net movement
Control	1.370438	-1.47625	0.8011
Mercury treated	0.986286	-1.90629	-0.9421

Figure 2: The table above shows the calculated averages of each directional movement and the total net movement of each of the 2 samples we were comparing in this experiment. For the control data that is used in this table we collected data from one area of the axons of one neuron. The control data included a total of 20 mitochondria that were measured

in that area. The Mercury treated data was collected from a different mitochondrion but was also in only one area of the axons. The mercury treated data included a total of 21 mitochondria that were measured in this area

A.



B.

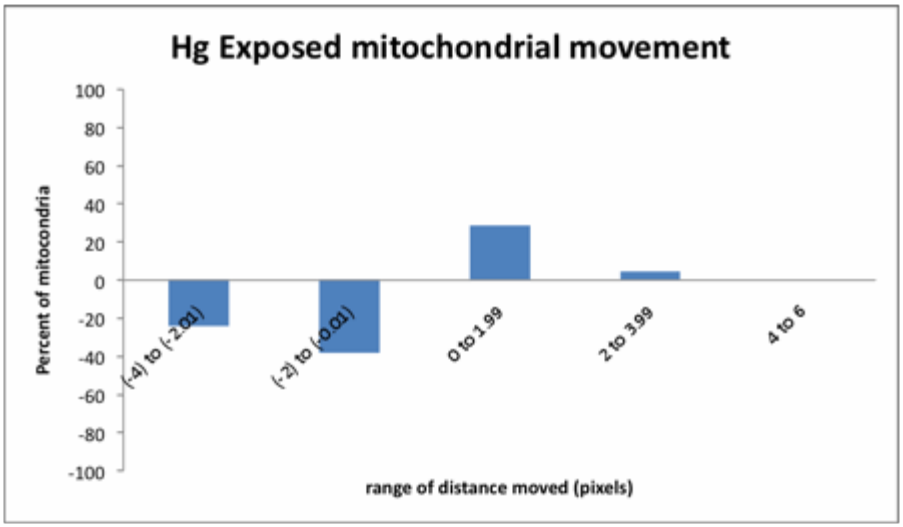


Figure 3: This figure shows the graphs of the percentage of mitochondria that showed each of the above divisions of movement. Graph A. shows the movement for the control sample, and graph B. shows the movement for the mercury-exposed sample. The two data sets from figure 2 were used again in figure 3 A. and B. Graph A. used the control set of data and graph B. used the mercury exposed set of data.

Discussion and Conclusions:

This experiment has supported our hypothesis that there would be an increase in retrograde movement seen in the mercury-exposed sample as opposed to the control sample. It should be noted that though there is a change in the

net movement and an increase percent of the mitochondria shown to move retrograde, there is still only about 1 pixel worth of movement. This is a small amount of movement that through further statistical analysis may not be significant. If this experiment was repeated 1000 times and the same data was shown this would in fact be strong support for our hypothesis.

This retrograde movement of the mitochondria may be caused by the decrease in the microtubule polymerase- causing there to be a lack of microtubules for the kinesin to carry the mitochondria down to the growth cones. Mitochondria start to move back towards the cell body because the higher levels of ATP in the growth cones are no longer needed when growth is impeded.

The major source of error in this experiment comes from the ability to measure substantial mitochondrial movement. The short time in between pictures left us with very little movement and unless we took a measurement of something like a speck of dust on the sample for some kind of stationary comparison we cannot definitively say that the movement seen is not just from human error. To improve upon this experiment, we will need to increase the time in between pictures taken to allow for more movement of the mitochondria. We should also in further experiments warm our samples while imaging because when not warmed the movement slows.

To extend our study in future experiments; it would be pertinent to look at changes in microtubule concentrations as well as doing further studies in the effect of mercury on concentrations of kinesin needed to move mitochondria down the microtubules. Such studies would be important because it will help further understand the exact processes that are being affected by the mercury exposure that leads to this change in the directional movement of the mitochondria.

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