Neurobiology 324 Home

Other research by Jasmine Bhatia

What are the effects of mercury on rates of axonal transport in neuronal primary culture chick sympathetic neurons?

Researched by Jasmine Bhatia
Posted at 5:46 AM on May 10, 2006
Bhatia_Jasmine@wheatonma.edu

I. Introduction

Mercury is a toxic metal found that humans frequently come into contact with. It can be found in the foods we eat and the water we drink, such is why it is important to test the effects of it on humans. 11 day old chick embryos were used as our model organism. At 11 days, chick embryos have neurons that have developed enough to be tested on but are still easy to identify and dissect. The use of their neurons is particularly relevant because chickens are vertebrates and as such they are closely related to humans. Furthermore, because they are embryos, we are able to see the possible effects of mercury on the chick embryo that could possibly occur in pregnant women who have been exposed to mercury whether through food or even in dental tooth fillings.

This experiment is adapted from the findings of Leong, Syed, and Lorscheider whose experiment is entitled "Retrograde degeneration of neurite membrane structural integrity of nerve growth cones following *in vitro* exposure to mercury." Leong et. Al. had the same purposes in exploring this topic, as mercury is a toxic substance that is present in the human body. They concluded that exposure to mercury disrupts the membrane structural integrity of neurites and growth cones. The concentration of mercury used is relevant as it is the EPA's recommended weekly intake.

The hypothesis put forward is that levels of axonal transport in mercury treated axons are lower than levels in control axons. Axonal transport is a process wherein materials are either brought toward the cell terminal or away from it in anterograde or retrograde fast transport, a form of transport that moves faster than 400 mm/day in warm blooded animals. Fast axonal transport carries large membranous organelles such as mitochondria or autophagic vacuoles. Fast axonal transport depends on a variety of factors. Anterograde transport depends largely on the microtubules that are the track that organelles can move by the means of molecular motor molecules called kinesin and KIFs, a large family of ATPases that transport different cargoes. Retrograde transport towards the cell body usually consists of endosomes

generated by endocytic activity at the nerve terminals. The rate of retrograde fast transport is actually one half to two thirds that of anterograde fast transportation. The motor molecule for retrograde fast transport is the microtubule associated ATPase called MAP- 1C. It can be compared to the dyneins in cilia and flagella (Kandel, 2000).

In anterograde transport, the motor molecules are kinesin and an array of kinesin related proteins called KIFs. KIFs are a large family of ATPases, each of which transports different membrane cargoes. Kinesin is a heterotetramer composed of two heavy chains and two light chains. The heavy chains contain a globular head that is the ATPase domain. This domain acts as the motor when attached to the microtubules. A coil coiled helical stalk is responsible for dimerization with the other heavy chain, and a C terminus interacts with the light chains and represents the organelle interaction domain. It was believed that kinesin moves organelles by walking along the microtubules, however recent studies have found that there are monomeric KIF motors with one foot (Kandel, 2000). Anterograde transport is used in the translocation of membranous organelles, such as mitochondria, as well as macromolecules, such as actin, myosin, clathrin, and some enzymes necessary for neurotransmitter synthesis at the axon terminals that are mediated by kinesin-family proteins (Oztas, 2003).

Retrograde transport in the axon move from the nerve endings to the cell body. Retrograde transport involves primarily endosomes generated by endocytic activity at nerve terminals, mostly multivesicular bodies and other endosomes as well as mitochondria and elements to the endoplasmic reticulum. Although much of this material is degraded within lysosomes, retrograde transport is also used to deliver signals to the cell body. Certain toxins as well as pathogens are transported toward the cell body along the axons. The molecular motor responsible for retrograde transport is a microtubule associated ATPase called MAP-1C. It is similar to dyneins in cilia and flagella and is consisted of a multimeric protein complex with two globular heads on two stalks connected to a basal structure. The globular heads attach to microtubules and act as motors moving toward the minus end of the polymer and like kinesin, the rest of the complex is thought to associate with the organelle being moved.

My collaborator is Stephanie Cummings who is studying the classes of fast axonal transport. Her studies would give us further knowledge as to the effects of mercury on fast axonal transport. The research of anterograde and retrograde transport might give us an idea as to what materials are being transported to and away from the cell body. My collaborator could also study the different types of organelles being transported along the axon to observe the state of the cell.

The study of axonal transport gives us insight into the secretory processes of neurons when a toxin such as mercury is introduced. Observation of fast axonal transport rates in neurons gives us insight into the distress of the cell. We can observe whether or not the cell is undergoing trauma from the mercury. These studies are particularly relevant

because the results could help us to reveal the effects of human exposure to mercury, a harmful substance that is present in the food we eat and the water we drink. Mercury is known to have a harmful effect on organisms and our research will show the extent to which humans are exposed to mercury and its effects on our health.

II.Materials & Methods:

11 day old chick eggs, 90% Ethanol/water, 30 mL HBSS (HankÕs Balanced Salt Solution), HCl- 0.5% in HBSS, 10 nm Methylmercury ($HgCl_2$) in HBSS solution in 0.5% HCl, Laminin, Poly-L-lysine, F+ growth medium, 10 µl Rhodamine 123, (3) 35 mm Petri dishes, (2) 110 mm Petri dishes, (2) slides, (2) coverslips, (10) Sterile Pasteur pipettes (2) Pasteur pipette bulbs, (2) Forceps (sharp and blunt), Coverslip chips, Kim wipes, Tinfoil, Rubber gloves, Stage heater, Waste bowl, Dissection scope, Nikon Eclipse E200 microscope (with fluorescent capability, Spot Advanced software

Primary Cell Culture of 11 day old chick embryos

A Pasteur pipette was flame-drawn. Using 11 day old chicken eggs, the blunt end was sterilized by spraying it with ethanol. While drying, a 110mm Petri dish was set up with about 5 mLs of warm Hanks Balanced Salt Solution in it. When the egg was dry, sterile dull forceps were used to tap through the blunt end and the top of the shell was lifted away. With forceps, the embryo was lifted out of the egg and placed in HBSS and the head was separated from the trunk. The head, wings, legs and viscera were removed from the body and discarded. The embryo was set down with the ventral side up and tissue was removed to expose the spinal cord. Dorsal root ganglia were removed as well as sympathetic chains that were lifted away. The dissected ganglia were placed in a 25 mm Petri dish with HBSS. Poly-L-lysine was used to coat the surface of two coverslips and was incubated for 1 hour. They were then rinsed with distilled water. Each coverslip was coated with a solution of laminin in HBSS for 1 hour and were then rinsed with HBSS and were placed in 3mL of growth medium. For our 1x density of neurons, 1 DRG was plated on each coverslip. The coverslips were left to incubate at 37C for 36 hours.

Making the Rhodamine 123 working solution

A 1 mg/ml solution of Rhodamine 123 in DMSO was obtained. A commonly used concentration of 1:1000 Rhodamine 123 to F+ growth medium was used, and thus 5 µl of Rhodamine 123 were added to 5 ml of F+ growth medium in a test tube. The test tube was then covered with tin foil to prevent photo bleaching.

Mercury Treatment

Two coverslips with 1x density of neurons were obtained. The growth medium was removed from dish A (experimental) and dish B (control). The 10 nm Mercury+ HBSS solution in 0.5% HCl solution was added to dish A

and 0.5% HCl + HBSS was added to dish B. Both dishes were incubated for 20 minutes. Hg+ HBSS was removed from dish A 0.5% HCl+ HBSS was removed from dish B. The mercury was disposed in the appropriate container. F+ medium with 0.1 µg/mL of Rhodamine 123 stock was added to both dishes. The working concentration of R123 was 1 µl/ ml. Both dishes were covered and incubated for 10 minutes at 37 degrees C. All R123 was pipetted out and each dish was washed once with HBSS. Chip chambers for coverslips A and B were prepared on separate slides using one drop of HBSS from the Petri dish.

Taking Fluorescent Images

The stage heater was turned on. Using a Nikon Eclipse E200 microscope and Spot Advanced software an in focus image of the desired axons were located. The bino/photo pin was pulled, and the "Live" button in the Spot Advanced program was pressed to get the image viewed on the computer. The transmitted light was blocked with a sheet of tinfoil, and the live image was turned off. The Spot Advanced program was closed, then reopened. The camera was switched to the Rhodamine 123 setting and the slider was moved to position 3. The shutter was opened immediately and closed after exposure. The slider was moved back to position 1.

Quantification of Rates of Axonal Transport

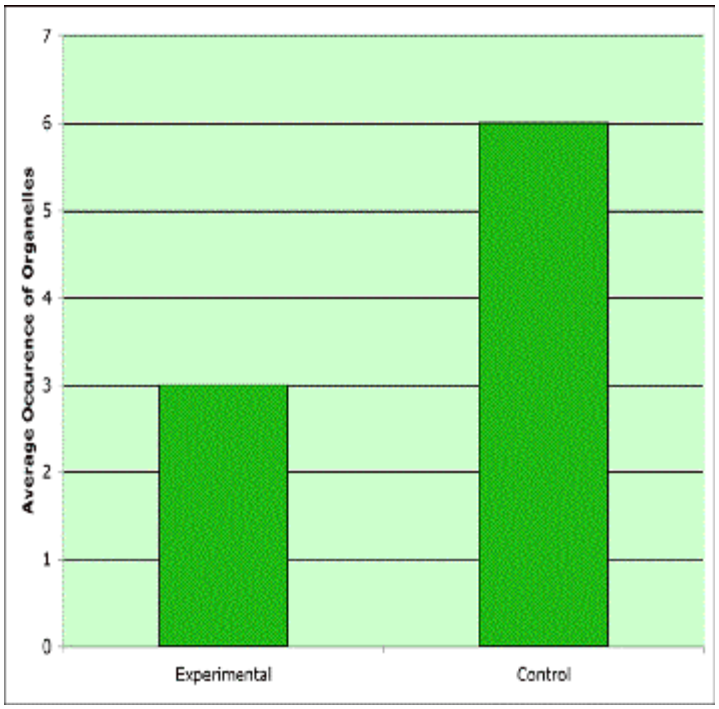
6 time lapse images at 100x with oil inversion were taken at intervals of 2 minutes. Single, distinct axons were located. These axons did not overlap with other axons, and did not connect to other axons, they simply extended away from the cell body undisturbed. A single point was chosen approximately in the middle of the axons, and the number of dark spots passing a certain point were counted for the duration of each movie. It is important to note that once a spot was chosen, its distance from the cell body was used in the rest of the axons to quantify the data so that similar areas could be compared for both the control and experimental. Dark spots are defined as any dark region that passed the stationary point, whether the movement be anterograde or retrograde. The totals for control and experimental were averaged and were displayed in a simple bar graph (refer to Figure 1).

III. Results

Trial	Control	Experimental
1	8	1
2	7	3
3	3	5
Average	**6**	**3**

Table 1. The Control group showed a higher amount of axonal transport as compared to the mercury exposed experimental group that showed half the amount of axonal transport.

Movies of both control and mercury exposed neurons were taken over a 2 minute and 55 second period. The occurrences of organelles passing a certain point are represented in the totals which are considered a trial. Each of the three trials for both groups were added up and divided by three to take an average.



Graph 1: The presence of mercury decreased the amount of axonal transport in neurons treated with mercury. The occurrences of organelles passing a certain point on similar specific regions on neurons were counted, and the mercury treated axons were found to be infrequent as compared to the control.

The averages calculated in Table 1 are the data shown in this graph. The averages were The control group shows double the amount of organelle transport than the experimental group. The two groups were graphed on the y-axis of average occurrence of organelles.

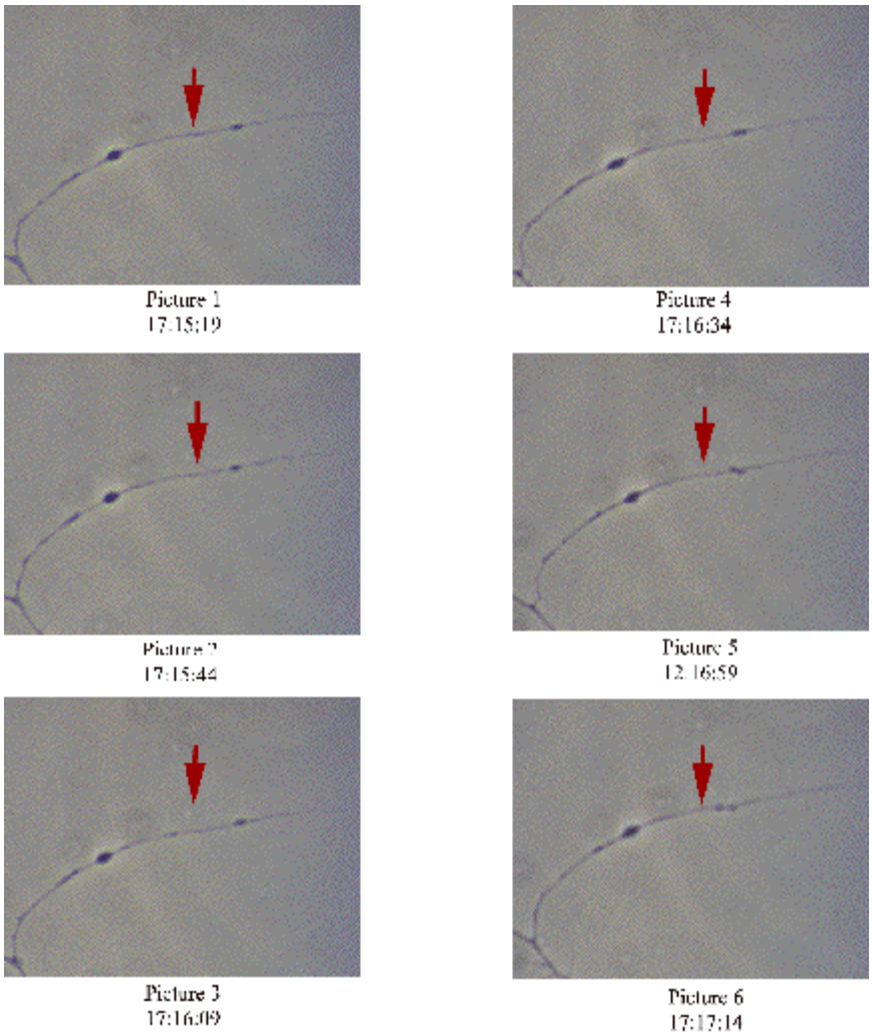


Figure 1: The stationary point technique is used on this sequence of control photos. A point is chosen and the number of organelles that pass it are counted. Once can note the approach of an organelle moving from the right to the left. The time is shown to show the progression of time, however this is not taken into consideration when counting the number of organelles.

IV. Discussion and Conclusions

These preliminary findings suggest that the amount of organelle transport is reduced in axons treated with mercury compared with non-treated axons. Thus, the hypothesis that the amount of axonal transport in mercury treated axons are lower than levels in control axons was supporting by these initial findings.

A likely explanation for the reduced amount of organelle transport in axons treated with mercury is due to the lack of microtubules. The amount of axonal transport is decreased because of the smaller amount of microtubules, on which organelles can move along, in mercury treated cells. Transport in the axons relies on microtubules that serve as a stationary track on which various organelles can move by means of molecular motors. As previously mentioned, there are two types of axonal transport, anterograde and retrograde.

Microtubules are dynamic structures composed of a single globular protein called tubulin. Tubulin is a dimer

that consists of two closely related polypeptides, a tubulin and b tubulin. These dimers polymerize to form microtubules and depolymerize to dissociate them. These rapid cycles of assembly and disassembly make up the dynamic instability of microtubules. Whether a microtubule grows or shrinks is determined by the rate of tubulin addition relative to the rate of GTP hydrolysis. As long as new FTP bound tubulin molecules are added more rapidly than GTP is hydrolyzed, the microtubule will retain a FTP cap at its plus end and microtubule growth continues. However, if the rate of polymerization slows, the GTP bound to tubulin at the plus end the microtubule will be hydrolyzed to GDP, and the GDP bound tubulin will dissociate resulting in rapid depolymerization and shrinkage of the microtubule. This is because GTP bound to the b tubulin undergoes hydrolysis during or shortly after polymerization, which reduces its binding affinity for adjacent molecules. The GTP is hydrolyzed more rapidly than new subunits are then the microtubules disassembles and shrinks (Cooper, 2004). The Leong et. Al. paper states that microtubule metabolism is compromised in the presence of mercury ions because it inhibits GTP nucleotide binding to b tubulin. They believe that the mercury-induced disassembly of microtubules is manifested in the observation of neurite membrane degeneration at growth cones with the exposure of mercury. Such a comparison can be made with the axons that were studied since they too are comprised of microtubules (Leong et. Al., 2000).

A lower amount of transport in neurons is profound. With decreased transport, the neuron cannot interact with neighboring cells effectively. This could have drastic effects on behavior and function in an organism. Deprivation of important proteins and materials is due to a lesser amount of axonal transport and can lead to degeneration of neurons and possibly the brain, depending on the amount of mercury present in the organism.

My collaborator Stephanie Cummings found that mercury effects mitochondrial metabolic activity. She observed that mercury treated axons were lower in metabolic activity than control axons. Our two studies are related in that metabolic activity in the axons can be regulated by axonal transport. Depending on the rate of transport, the metabolic activity can be effected. She used Rhodamine 123 staining to quantify the metabolic activity of mitochondria through brightness.

This experiment was successful in that many trials were conducted, however more trials could have been conducted so as to ensure that the data being collected are consistent with the methods. These data are still preliminary in that further studies could have been done to specify which organelles were being transported. A future study could be conducted regarding the effects of mercury on the rates of anterograde versus retrograde transport in the axons. Also, there could be further studies done that might explore the amounts of specific organelles being transported because perhaps when exposed to mercury, the amount of transport of one organelle might remain the same while the amount of another type of organelle might be lower. To refine this experiment and attain more accurate results, one could observe

a single neuron before mercury exposure and after to observe the effects by flowing in a mercury solution to a neuron. These results might be more accurate as they would measure the same neuron before and after mercury exposure rather than six different neurons that may have inadvertently been exposed to different conditions. Due to time constraints, we were unable to prepare each neuron one at a time, so mercury might have even more of an effect on axons when exposed for a greater amount of time. Each axon should be observed after a consistent amount of time. By preparing each neuron and collecting data immediately, one might be able to obtain more accurate results as mercury could possibly decrease the rate over time, a possibility that could potentially make averages unreliable.

V. Bibliography

Chick, Brittany, personal communication 2006

Cooper, Geoffrey M., Hausman, Robert E., (2004) *The Cell- A Molecular Approach*; (435- 589)

Cummings, Stephanie, personal communication 2006

Hollenbeck, Peter J., Morris, Robert L. *Primary Culture of Chick Embryonic Peripheral Neurons*

Kandel, E. R., Schwartz, J. H., & Jessel, T. M. (2000). *Principles of Neural Sciences* (4th ed.). New York: McGraw Hill. (Original work published 1991)

Jeffrey, Brianne, personal communication 2006

Kovacs, Esther, personal communication 2006

Kyes, Danielle M. (2004). Mitochondrial Density in Regions of Axonal Overlap Compared to Axonal Regions Distal to Overlap <http://icuc.wheatonma.edu/%7Ebio324/dkyes/index.html> [Accessed March, 2006]

Leong, Christoper C.W., Syed, Naweed I., Lorscheider., Fritz L. (2000) Retrograde degeneration of neurite membrane structural integrity of nerve growth cones following *in vitro* exposure to mercury *Neuroreport* 12:733-737

Morris, Robert L., personal communication 2006 Oztas, Emin (2003) Neuronal Tracing *Neuroanatomy* 2:2-5

Wikipedia. Axoplasmic Transport. 3 March 2006. <http://en.wikipedia.org/wiki/Axonal_transport>. [Accessed April, 2006]