Evidence for Mercury-induced Decrease in Activity of Mitochondria Found in the Region of Axon Extending 30µm from the Neuronal Growth Cone

Liz McKay
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Introduction

Neurological function is essential to human life. From the brain, we rely on regulation of breathing, muscle coordination and a whole host of motor and sensory activity to survive (Scanlon & Sanders, 2007). At the cellular level, the study of neurons is important in characterizing the health of the peripheral nervous system (PNS) (class notes, 01.24.08). Specifically, the examination of mitochondrial activity and membrane potential heterogeneity is central in assessing the health of a growing neuron. As a dynamic site of growth, the growth cone requires a tremendous supply of energy in the form of ATP to drive biochemical reactions in cell metabolism (Morris, personal communication, 2.6.08). Mitochondria, often referred to as the “powerhouse” of the cell, provide this ATP energy (Scanlon & Sanders, 2007). If the mitochondria of a given neuron are in any way compromised during cell metabolism, they may be unable to provide an adequate amount of ATP and consequently inhibit neuronal growth.

In our present day environment, there are many harmful chemicals and toxins that threaten the proper functioning of regulatory systems such as the nervous system. Mercury (Hg), one such toxin, is a naturally occurring metal and according to the Environmental Protection Agency (2006) is continually released into the Earth’s atmosphere from natural and anthropogenic sources. The main anthropogenic source of mercury comes from coal emissions (Environmental Protection Agency, 2006). Beyond atmospheric contamination, this metal resides in water and much of the food we eat (Zahir et al., 2005). Because it is ubiquitous in our environment, and because according to the Agency for Toxic Substances and Disease Registry (2007), “the Nervous System is sensitive to all forms of mercury,” there is growing interest in researching its role in neurological disorders.

Today, there is increasing concern that mercury may be involved in a number of neurological diseases. The effects of mercury on the human brain and its neurological function are speculated to be a contributing factor in many neurodegenerative disorders such as Alzheimer’s disease, attention-deficit hyperactivity disorder (ADHD) and Autism Spectrum Disorders (Geier & Geier, 2004; Deth et al., 2007). If mercury weakens mitochondrial membrane potential, this in turn compromises the organelle’s ability to generate and deliver ATP to the neuron. By investigating the effects
of this naturally occurring metal teratogen on the electrochemical makeup of the mitochondrial membrane, one can begin to infer the damage caused by insufficient ATP supply to the growth cone of neurons.

In this study, sympathetic nerve chains and dorsal root ganglia were dissected from the Peripheral Nervous System of 9-11 day old chick embryos, *Gallus gallus* (Morris, 2008). Neurons were obtained and studied by dissociating dissected cells. Within these neurons, mitochondria were chosen as model organelles to study organelle transport and growth of neurons because they are easily identifiable and their transport involves microtubules and actin. In addition, they undergo antrograde and retrograde movement and mobilize and respond to physiological demands of the cell (Hollenbeck *et al.*, 2005).

JC-1 dye was used to visualize changes in the electrochemical membrane potential of the mitochondria. The formation of J-aggregates occurs via polymerization (Morris, personal communication, 3.28.08). The large size of these aggregates causes them to diffuse slowly through the cell and its organelles, thus making them useful as reporter molecules for localized biochemical events (Smiley *et al.*, 1991). A study involving JC-1 and mitochondrial membrane potentials reveals that “…J-aggregates may serve as reporter molecules for heterogeneity in mitochondrial membrane potentials in living cells” (Smiley *et al.*, 1991). This dye was chosen over rhodamine-123 because it separates membrane charges by color based on strength; green dye indicates weakly-charged membranes and red indicates strongly-charged membranes. From this dye, I was able to quantify the relative brightness of red and green mitochondria used in assessing the health of the growing neuron under mercury exposure.

In this Independent Research Project, I studied the effects of ionic mercury on mitochondrial activity in regions proximal to the growth cone of a growing axon using JC-1 vital staining and still-frame fluorescence imaging (class notes, 4.10.08). I hypothesized that mercury would decrease the activity of mitochondria occupying the region of axon extending 30µm from the end of the growth cone as observed in weakened membrane potential. In my research, I collaborated with Suzanne Frasca. In her experiment, Suzanne quantified the distribution, and specifically the number of mitochondria found in the region of axon extending 30µm from the growth cone in the presence of ionic mercury using JC-1 and ignoring color difference (Morris, 1993). The goal of our respective projects was to test for the toxicity of ionic mercury on mitochondrial activity and abundance, thus impacting the overall health of the growing neuron.

If ionic mercury does in fact disrupt mitochondrial membrane potential this could have important clinical application in remedying neurodegenerative disorders. In addition, mercury-induced damage to neuronal mitochondria could have implications in environmental policy when considering the already large abundance of the metal in our atmosphere and its current role as a potentially harmful teratogen in human development.
Materials & Methods

Materials:

Sharp sterile forceps, 110mm Petri dishes, latex gloves, paper towels, an empty egg crate, Kim wipes, 90% ethyl alcohol (EtOH), and a waste beaker were used to explant 9-11 day old fertilized chick embryos.

A dissection light microscope, blunt and sharp sterile forceps, Hanks Balanced Salts Solution (HBSS), sterile Pasteur pipettes, Pasteur pipette bulbs, a waste beaker and 25mm Petri dishes were used to dissect sympathetic nerve chains and dorsal root ganglia from 9-11 day old fertilized chick embryos. A 37 degree Celcius incubator was used to house all successfully explanted cells (Hollenbeck, 1988).

25mm sterile coverslips, 1mg/mL poly-L-lysine (poly-K), sterile Pasteur pipettes, 110mm Petri dish, laminin in HBSS and HBSS were used to prepare a laminin/poly-K substratum for dissociated cells (Hollenbeck, 1988).

HBSS, sterile Pasteur pipettes and bulbs, trypsin solution (Ca/Mg-free HBSS containing 0.25% trypsin), 37 degree Celcius incubator and a sterile drawn Pasteur pipette were used to dissociate sympathetic nerve chains and dorsal root ganglia (Hollenbeck, 1998).

Sharp sterile forceps, coverslip chips, a microscope slide, growth medium solution, Kim wipes, electric heating element, a paint brush, VALAP, deionized water, sterile Pasteur pipettes and bulbs, and a coverslip of dissociated cells were used to create a ‘chip chamber’ (Neurobiology Laboratory Notebook, 2.14.08, p.67, EAM).

Sterile Pasteur pipettes and bulbs, HBSS, L-15 solution, DMSO, JC-1, tin foil, vortex tubes, VALAP, a waste beaker, Kim wipes, ionic mercury (HgCl₂) and a sharp sterile forceps were used to prepare control JC-1 and mercury-treated chip chamber-prepared light microscope slides, respectively (Neurobiology Lab Notebook, 2.14.08, p.67-69, EAM).

Using the Imaging Center for Undergraduate Collaboration (ICUC), the “Capricorn” computer, the “Capricorn” fluorescence light microscope, a stage heater, Advanced Spot software, Image J software, a Nikon SMZ-660 camera, a ruler, the “Capricorn” camera adapter C Mount objective 1.0x magnification and a piece of Styrofoam were used to image fluorescing mitochondria (www.icuc/wheatonma.edu).

Methods:

A modified version of the Hollenbeck (1998) experimental procedure was used to perform a primary culture of 9-11 day old chick embryos. Sympathetic nerve chains and dorsal root ganglia were dissected from the peripheral nervous system. Prior to explanting, all bench top surfaces were sterilized using 90% ethyl alcohol (EtOH) using paper towels. Fertilized, 9-11 day old chick embryos were removed from the 37 degree Celsius incubator and candled to determine
viability.

Primary Chick Culture of Peripheral Sympathetic nerve chains and Dorsal Root ganglia

Explanting the chick embryo into the Petri dish was performed and demonstrated by Professor Morris. All eggs were sterilized by Professor Morris using paper towels and 90% EtOH. Holding the egg narrow end down low over a waste beaker, the blunt end of a forceps was used to pierce the shell. The forceps were used to chip away at the shell (Hollenbeck, 1998). Next, Professor Morris removed the chick embryo from its shell by grasping the neck between the forceps and placing it into a 110mm Petri dish containing about 5mL of HBSS (Neurobiology Lab Notebook, 2.12.08, p.59, EAM). Professor Morris then separated the head from the remainder of the body and removed the wings and legs by pinching them off with the forceps.

Each student was given a chick torso. Carefully, the remaining torso was flipped onto the ventral side using both sets of forceps. Through a series of steps dictated by Professor Morris, each student removed the body tissue to expose the spinal cord (Neurobiology Lab Notebook, 2.5.08, p.39, EAM). Periodically, HBSS was squirted into the dish using sterile Pasteur pipettes with bulbs to better visualize the spinal cord. Once identified under the light microscope, the sympathetic nerve chains were removed by using the blunt forceps to stabilize the torso and the sharp forceps to lightly brush over the spinal cord. With some luck, the nerve chain was isolated and transferred into a 25mm Petri dish with HBSS (Hollenbeck, 1998). Finally, the dorsal root ganglia (DRGs) were removed by stabilizing the torso with the blunt forceps and pinching off the dark balls studding each side of the spinal cord with the sharp forceps (Neurobiology Lab Notebook, 2.5.08, p.39. EAM). The dorsal root ganglia were then transferred into the same 25mm Petri dish as the sympathetic nerve chain.

Preparation of substrata

To prepare a substratum for the dissociated nerve chain and DRG cells, coverslips were placed in 25mm Petri dishes and treated with 90% EtOH to sterilize (Neurobiology Lab Notebook, 2.5.08, EAM). A Kim wipe was used to wipe the front and back sides of the coverslips allowing each to dry before baking. Droplets of poly-L-lysine (Poly-K) were placed onto a 110mm Petri dish and baked coverslips were then placed on top of each droplet and incubated anywhere between 1 and 24 hours (Hollenbeck, 1998; Morris, personal communication, 4.10.08). Coverslips were rinsed with deionized water and left to dry for 20 minutes. Laminin was then applied to each coverslip and left to incubate for anywhere between 1 to 24 hours (Morris, personal communication, 4.10.08). Following incubation, laminin-treated coverslips were rinsed with HBSS and kept in the Petri dish to remain wet (Neurobiology Lab Notebook, 2.5.08, EAM).
Cell dissociation of Peripheral Sympathetic nerve chain and Dorsal Root ganglia

For each week of experimentation, Professor Morris dissected nerve chains and DRGs and performed cell dissociation. HBSS was used to wash nerve chains and DRGs two times in preparation for dissociation (Hollenbeck, 1998). HBSS was then replaced with trypsin, a protease cocktail solution to degrade all proteins associated with extracellular matrix and transferred to the 37 degree incubator for 20 minutes (Neurobiology Lab Notebook, 2.5.08, p.45, EAM). Carefully, Professor Morris removed the trypsin using a Pasteur pipette without sucking up cells. HBSS was added back into the dish and a drawn Pasteur pipette was used to triturate the solution to dissociate the cells (Hollenbeck, 1998). The density varied depending on the number of DRGs dissected. For instance, in week 1, the density was 18 DRGs, 24 dishes, and 1 DRG per dish with 1mL of growth medium (Neurobiology Lab Notebook, 2.19.08, p.70, EAM). Once density was calculated according to week, the appropriate number of dissociated cells was introduced into a 25mm Petri dish containing L-15 medium with prepared coverslips. All dissociated cell dishes were returned to the 37 degree Celcius incubator and left to grow out over a period of 19-25 hours (Neurobiology Lab Notebook, 3.18.08, p.88, EAM).

Preparation of Mercury and JC-1 solutions

Mercury was obtained from Jani Benoit, Professor of Chemistry. To prepare 40mL of a10nM working solution, Professor Morris diluted 0.04mL of ionic mercury (HgCl2) stock solution in 40mL of HBSS (Neurobiology Lab Notebook, 3.4.08, p.83, EAM).

JC-1 dye was prepared by Professor Morris at the beginning of each day of data collection. JC-1 was originally 5mg of fine powder (Professor Morris, personal communication, 3.4.08). To prepare a 1μg/mL solution, 2.5mL of dimethyl sulfoxide (DMSO) was added to this powder for a final concentration of 2mg/mL (Neurobiology Lab Notebook, 3.4.08, p.83, EAM). The solution was diluted 1:2 by adding 0.5mL DMSO with 1.5mL of 2mg/mL JC-1. A 1:1000 dilution was performed by adding 5μL of JC-1 to 5mL of Growth medium, arriving at a final concentration of 1μg/mL JC-1 (Neurobiology Lab Notebook, 3.4.08, p.83, EAM).

Preparation of Dissociated cells for Microscopic analysis

Fluorescence Microscopy was used to observe any differences in mitochondrial membrane potential in the
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presence of mercury. For controls, with no exposure to mercury, dissociated cells were first removed from the 37 degree Celcius incubator in the Lab Prep room. HBSS was removed from the Petri dish using a Pasteur pipette and replaced with JC-1 prepared in 1:1000 dilution with a final concentration of 1µg/mL (Neurobiology Lab Notebook, 3.4.08, p.83, EAM). Incubation times for JC-1 and mercury were followed and modified according to Bhatia (2006). Dissociated cells were covered with tin foil to prevent bleaching and incubated with JC-1 dye for 5 minutes in the 37 degree Celcius incubator (Bhatia, 2006). Once this time had elapsed, the cells were washed (x3) with either HBSS or L-15. Then, cells were washed (x1) with Growth medium (Neurobiology Lab Notebook, 3.4.08, p.85, EAM). For experimentals undergoing mercury exposure, dissociated cells were first treated with 10nm of ionic mercury (HgCl2) for 20 minutes (Bhatia, 2006). To accomplish this, HBSS was almost entirely removed from the 25mm Petri dish before covering the coverslip with ionic mercury. Following incubation, the ionic mercury was removed from the cells by washing with HBSS or L-15 (x3) (Neurobiology Lab Notebook, 4.1.08, p.119, EAM). Cells were then treated with approximately 1mL of JC-1 dye and incubated under the same conditions as the controls. Following incubation, cells were washed in the same manner as the controls. In the case of controls and experimentals, one drop of Growth medium was placed in the center of a chip chamber to prepare the cells for slide contact.

In order to visualize neurons under the light microscope, it was necessary to prepare dissociated cells in a ‘chip chamber’ (Neurobiology Lab Notebook, 2.14.08, p.67, EAM). Chips were prepared by crushing one coverslip wrapped in a Kim wipe. Chips were deposited into a 25mm Petri dish. With forceps, my lab partner, Suzanne Frasca and I transferred approximately one dozen chips onto a microscope slide and oriented them in a circle. For both controls and experimentals, coverslips of dissociated cells were carefully removed from the Petri dish using a forceps. Oriented with the cells (side up), the bottom surface of the coverslip was wiped using a Kim wipe (Neurobiology Lab Notebook, 2.14.08, p.67, EAM). Still grasping the coverslip with a forceps (side up), the coverslip was rotated 180 degrees and placed (side down) on top of the drop of Growth medium in the center of the chip arrangement. Any excess liquid was removed or added using a Pasteur pipette. Following this, warm VALAP was used to coat the edges of the coverslip and create a seal (Neurobiology Lab Notebook, 2.14.08, p.67, EAM). Deionized water was used to wash the salt from the surface of the coverslip. Kim wipes finished the preparation of this chip chamber slide as they were used to wipe the surface (Neurobiology Lab Notebook, 2.14.08, p.67, EAM).

All fluorescence images were taken using the “Capricorn” fluorescence microscope and computer in the Imaging Center for Undergraduate Collaboration (ICUC). To capture an image, neurons were first focused using the microscope (Neurobiology Lab Notebook, 3.4.08, p.85, EAM). The software program, Advanced Spot was used to capture phase and fluorescence images. Once focused, a ‘phase’ image was captured and saved. Following this, red and green
fluorescence images were taken and saved. This procedure was used for both controls and experimental (Neurobiology Lab Notebook, 3.4.08, p.85, EAM). The freeware program, Image J was used to analyze data. Histograms were generated to measure average brightness of red and green mitochondria in the region of axon extending 30μm from the growth cone (Neurobiology Lab Notebook, 4.3.08, p.127, EAM). Ratiometric images were also created in Image J to visualize mitochondrial activity on a full cell scale (Neurobiology Lab Notebook, 4.3.08, p.131, EAM).

Data Collection Time frame

Data collection was performed on a weekly basis for approximately 3 hours. Data were gathered in three trials (Neurobiology Lab Notebook). At the beginning of each session, chip chambers were prepared and dissociated cells were incubated with either JC-1 (controls) or ionic mercury followed by JC-1 (experimentals) and left to sit and acclimate for one hour prior to imaging (Neurobiology Lab Notebook, 4.1.08, p.119, EAM). Controls were a vital component of this experiment. They allowed us to visualize mitochondrial activity under normal conditions. The activity of the mitochondria here, then gave us a reference from which to hypothesize the possible changes in activity the mitochondria would exhibit in the presence of ionic mercury. Thus, the controls were both necessary and appropriate for this study.

At the conclusion of data collection, all data were analyzed using Image J. Those images that most easily allowed measurement mitochondrial activity in the region of axon extending 30μm from the growth cone were used (those with relatively straight axons) (Neurobiology Lab Notebook, 4.3.08, p.133, EAM). Averages were taken for mean brightness among red control mitochondria, as well as green control mitochondria. Averages were also taken for experimental red and green mitochondria. Once this was completed, the average background brightness for each image was measured again using the histogram feature in Image J. Then, each measured background was subtracted from each image. The average background brightness was then subtracted from the average brightness for each image of red or green mitochondria. In total, there were 3 neurons used for red mitochondria and 3 neurons for green mitochondria under controlled conditions. There were 3 neurons used for red mitochondria and 3 neurons used for green mitochondria under experimental conditions (Neurobiology Lab Notebook, 4.3.08, p.133, EAM). All averages calculated by Image J were measured in pixels. A stage micrometer was used to convert pixels into micrometers (Morris, personal communication, 4.1.08).

Results

The following images serve to illustrate the ‘phase image’ of neurons under control and experimental conditions. These
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Phase images were used as a guideline for measuring mean brightness in the region of axon extending 30µm from the growth cone for red and green mitochondria images (Neurobiology Lab Notebook, 4.3.08, EAM).

Figure 1: Phase Image for Control neuron. This image represents a typical neuron growing in the absence of mercury. Notice the small, dark spots in the axon as you move away from the cell soma, these are mobile mitochondria.

The ‘Stacks’ feature in Image J was used to generate a slideshow of phase images in sequence with their corresponding red and green images. Phase images were used to define the end of the growth cone. The arrow on Figure 1 indicates the end of the growth cone and the beginning of the axon. Based on personal communication with Professor Morris, my lab partner and I defined the beginning of parallel lines, forming the sides of the axon, as the end of the growth cone (Morris, personal communication, 3.4.08). In Figure 1, the arrow marks the end of the growth cone and the box is proportionate to 30µm of measured area extending down the length of the axon.

The following phase image was again used as a reference in defining the end of the growth cone under experimental conditions.
**Figure 2:** Phase image for experimental neuron. This image is an example of a neuron in the presence of 10nM of mercury (HgCl$_2$). To locate mitochondria, look closely at the small dark spots running the length of the axon. Notice the glial cell located at the bottom left-hand corner of the image.

The phase image was used to demarcate the end of the growth cone and the beginning of the axon for purposes of measurement. Interestingly, this neuron has two branched axons extending away from the cell soma.

The following graph was generated to compare average brightness between red and green control and experimental mitochondria.
Figure 3: Average red and green mitochondrial brightness in controls and experimentals measured in the region of axon extending 30μm from the growth cone. Notice the difference in relative brightness between red and green mitochondria in the controls versus the experimentals. Over the course of three trials, n=3 neurons for controls, and n=3 neurons for experimentals.

Figure 3 demonstrates the difference in average brightness under control versus experimental conditions. In the absence of mercury, red mitochondria fluoresce more brightly than green mitochondria of weaker membrane potential. However, in the presence of mercury (the experimental condition), the relationship is inverted so that the green mitochondria fluoresce brighter than the red mitochondria of stronger membrane potential.

The following figures visually demonstrate the membrane potential activity of red and green mitochondria contained within a single neuron.
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Figure 4: Superimposed image of red and green mitochondria for control neuron. The background has been subtracted from both images before being superimposed to emphasize the activity of the mitochondria along the axon. Notice the large presence of red mitochondria extending away from the growth cone.

In Figure 4, there is a much higher presence of red mitochondria suggesting stronger mitochondrial activity in regions proximal to the growth cone.

Figure 5: Superimposed image of red and green mitochondria for experimental neuron. Notice the larger quantity of green mitochondria in the axon in the bottom right corner of the image.

As seen in Figure 3, the relationship between red and green relative brightness has been inverted between control
and experimental conditions. In Figure 5, we can see that the green mitochondria are, on average, fluorescing more brightly near the end of the growth cone than the red mitochondria.

Discussion

There is evidence from my data to support the original hypothesis that ionic mercury decreases the activity of mitochondria found in the region of axon extending 30μm from the growth cone as observed in weakened membrane potential. There is also evidence to support the notion that rate of JC-1 dye loading is slowed in the presence of mitochondria as observed in higher average brightness of green mitochondria (Morris, personal communication, 3.25.08). In order to appreciate this phenomenon, we must understand the mechanism of JC-1 entry into neurons. To begin, the JC-1 is introduced into the extracellular fluid surrounding the neuron (Morris, personal communication, 3.25.08). During incubation with the dye, the JC-1 dye moves into the cytoplasm by virtue of the neuron’s negatively charged membrane relative to the extracellular fluid. Since JC-1 is a positive, cationic molecule, it seems logical that this event would occur. Once the JC-1 has entered the cell, it then moves into the mitochondria again because they are now negatively charged relative to the cytoplasm (Morris, personal communication, 3.25.08). Initially, all mitochondria emit green light because JC-1 has entered through their lipid bilayer. Once a high enough concentration of JC-1 has collected into a mitochondrion, J-aggregates form and the organelle emits red light (Smiley et al., 1990). The JC-1 moves faster into mitochondria that have a stronger membrane potential and are thus more negative which creates a red light. In turn, the JC-1 forms J-aggregates more slowly in mitochondria with weaker membrane potentials, which explains why they remain green (Neurobiology Lab Notebook, 3.25.08, p.107, EAM).

Left for an extended period of incubation, the JC-1, originally a green dye, would eventually reach a high enough concentration in all mitochondria, aggregating by forming J-aggregates to emit red light (Morris, personal communication, 3.25.08). However, it is a delicate equilibrium of green and red mitochondria that must be achieved in order to analyze activity in the presence of ionic mercury. So, my lab partner, Suzanne and I chose a 5 minute incubation time. Following 5 minutes of incubation, we stopped labeling and washed out the JC-1 with HBSS or L-15.

With an understanding of the nature of JC-1 entry and polymerization at the cellular level, we can now examine the activity of mitochondria in the presence of 10nM ionic mercury. From this study, these data support the hypothesis that the activity of mitochondria is weakened in the presence of this metal, as observed in a slowed rate of JC-1 loading into the organelles. If this rate was slowed, it can be inferred that the dye encountered more weakly charged mitochondria or less strongly charged mitochondria during incubation (Neurobiology Lab Notebook, 3.25.08, p.107, EAM). By first exposing neurons to mercury, we eliminated any residual effects of mercury-induced changes in the JC-
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In both the controls and the experimentals, JC-1 was added only to visualize mitochondrial activity once one hour had elapsed following treatment (Neurobiology Lab Notebook, 4.1.08, p.119, EAM). In addition, my lab partner and I were successful in establishing an internal control by using the same concentration of JC-1 (1µg/mL) over the same incubation period of 5 minutes (Neurobiology Lab Notebook, 3.4.08, p.83, EAM). By using this length of incubation, we observed both green and red fluorescing mitochondria which indicated that we had not overshot the equilibrium of labeling the organelles (Figure 4, 5). Finally, the use of control mitochondria was critical in assessing the normal activity of mitochondria found in the region of axon extending 30µm from the growth cone. The control mitochondria, with the relatively higher brightness of red versus green mitochondria indicated more negatively charged mitochondria near the growth cone (Figure 3). Considering the energy needs of the growth cone, it seems logical that we would observe this ratio. However, in the presence of ionic mercury, there was a higher average brightness of weaker mitochondria near the growth cone (Figure 3). This result suggests that the rate of JC-1 loading was slowed in the presence of this metal. Thus, these data support the hypothesis that ionic mercury compromises the activity of mitochondria found in the region of axon extending 30µm from the end of the growth cone.

If this experiment were to be repeated one thousand times more with statistically significant results mirroring those in the present study, I would be apt to again conclude that ionic mercury is reducing the activity of mitochondria found in the region of axon extending 30µm from the growth cone. If there is a higher mean brightness of green mitochondria, this suggests that the dye is not collecting as quickly, consequently resulting in fewer red mitochondria. If I was fortunate to have a very large amount of data to support this same result, I would justify the result from a cellular perspective. Here, I would argue that the ionic mercury is weakening the mitochondrial membrane potential and thereby, causing the JC-1 dye to load much more slowly into the organelles. In turn, the higher brightness of green mitochondria would support this claim by suggesting that there are fewer strongly charged mitochondria for JC-1 to aggregate into, and thus, a lower brightness of red mitochondria.

In this research project, my lab partner and I had many sources of error (Frasca, personal communication, 4.3.08). Fortunately though, most of our error should have equally affected both control and experimental neurons (Morris, personal communication, 4.10.08). In two of the three trials, the JC-1 precipitated out of solution when injected into the HBSS by Professor Morris. As a result, we had to warm and gently rotate the liquid in an attempt to re-suspend some of the JC-1 particles back into solution. A second source of error was keeping the cells from bleaching once they had been exposed to JC-1. In an attempt to avoid bleaching, we tried to keep tin foil around the cells when we were not using them and during microscopic analysis. However, there was inevitably light penetrating into the cells which would have changed their properties during fluorescence imaging. During each trial, we tried to keep the

Microscope stage heated at 37 degrees Celsius, but this again was never held constant. Changes in temperature would have also either increased or decreased the activity of the mitochondria during imaging. There were additional errors committed during quantification. Not all images were used in data analysis because the majority of axons were too curvy to properly box. Further, the ‘polygonal selections’ tool we used on Image J only measured 168 pixels (equivalent to 30μm) on one side of the axon (Neurobiology Lab Notebook, 4.3.08, p.127, EAM). To try and standardize this technique, my lab partner and I decided to measure 168 pixels along the inside of the axon under examination (Frasca, personal communication, 4.3.08).

To refine this experiment, I would increase the n-value dramatically. In order for these or any results to be more convincing, there must be a large sample size. If one can observe a trend among six neurons and then observe the same trend in six hundred neurons, the results suddenly become much more compelling. From my knowledge, I am unaware of any other research done on this specific work. Therefore, it is difficult to compare my results with previous literature. However, it would be beneficial to measure a different segment of the axon in future research. Also, it would be interesting to examine mitochondria near the growth cone under varying concentrations of mercury to observe whether or not higher concentrations of mercury completely halt JC-1 loading.

In summation, this research project was successful in many ways. As a researcher, I was able to navigate new software such as Image J, Photoshop and Advanced Spot. By the end of the project, my lab partner and I were able to perform a primary chick culture of peripheral neurons, prepare chip chambers, visualize neurons under the light microscope, perform fluorescence imaging and measure mean brightness of mitochondria (Frasca, personal communication, 4.3.08).
Bibliography


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Morris, R.L., personal communication: 2.6.08, 3.4.08, 3.23.08, 3.28.08, 4.1.08, 4.10.08


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Lecture materials:

Class notes: 01.24.08; Jani Benoit, guest lecturer, 4.10.08

Internet sources:

Environmental Protection Agency: http://www.epa.gov/glnpo/bnsdocs/mercsrce/merc_srce.html#II.

Agency for Toxic Substances and Disease Registry: http://www.atsdr.cdc.gov/tfacts46.html#bookmark05