The Effects on Mitochondrial charged Regions in Chick Embryonic Peripheral Neuronal axons pre-Exposed to Mercury

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Introduction:

Mitochondrial charge is a very important aspect of the living cell. The mitochondrion provides the energy a cell needs to move, divide, produce secretory products, contract - in short, they are the power centers of the cell (Sullivan, 2011). In axons the growth of its process focuses on ATP, which is created by mitochondria. Mitochondria play a crucial role in living cells by producing ATP that is the basis of cellular energy and a key factor in cellular communication and differentiation as well as other cellular functions. (Ekizian, 2007). If a toxin like mercury is exposed to a cell it may disrupt the making of ATP in mitochondria and it is possible that the growth factors in axons will also be disrupted along with the Charge of a mitochondria (Morris, Hollenbeck 1993). Neurogenesis studies have shown that the growth of new neurons in the brain can be affected by mitochondria. Some recent studies have been done on mitochondrial involvement in neurogenesis, specifically on inflammation and how it can inhibit mitochondrial function (Voloboueva, 2011). In this study we used 10-day-old embryonic chick embryos and collected their DRG (dorsal root ganglia). Domesticated chickens, *Gallus gallus*, is an important and significant system to do this study on because its embryonic development is easily accessed and can be manipulated, thus explaining its historic importance as an embryological model (http://www.nih.gov/science/models/gallus/).

For our experiment we performed the process of degeneration in the DRG's by exposing the neurons to mercury and comparing it to the axons of a control that is exposed to a buffer (DMEM). My hypothesis was that mitochondrial regions in control axons have a higher charge than mitochondrial regions mercury exposed axons. One would expect this hypothesis to be true since in the Leong, Syed et al. 2000 article concluded that if mercury is added to neurons then their axons will be disrupted and it may activate the mitochondria within them, and that mercury disrupts membrane structures.

Materials:

* The materials below were performed and can be found in the *Primary Culture of chick Embryonic Peripheral Neuron* packet "Morris (2011a)". The Viewing and imaging data materials are an addition to the materials previously listed according to what was used for the experiment.

- Viewing and imaging data materials
  - SPOT Insight fire wire 2mega sample camera Model # 18.2 Color Mosaic diagnostic instrument Camera
  - Macintosh Desktop, Mac OS X Version 10.5.8, Processor 2 Ghz Intel Core 2 Duo (Capricorn Macintosh desktop in the Wheaton College ICUC of the Mars Center for Science and Technology building)
  - Microscope Nikon Eclipse E400
  - SPOT software Version 4.6 Imaging Solution, Copyright 2009
  - Image J software 1.40g, Wayne Rasband, Java 1.5.0_30 1142K of 400MB, Public Domain
  - Preview software Version 4.2 (469.5) Copyright 2002, Map imagery owned by NASA, 2007 Apple Inc.

Methods:

The first steps in the experiment were the dissection and primary culture of chick embryonic peripheral neurons found in “Morris (2011a)." Cells were then observed after incubation following the steps found in "Morris (2011b)."

Methods for our dissecting, staining, and imaging can all be seen in "Morris (2011a), Morris (2011c)" in the same order. The only step during the staining process that changed from the standard procedure for our control experiment was step nine, which calls for an incubation of ten minutes, but it was incubated for twenty minutes.

Methods for our experimental were similar to our control but we added mercury which would be the step after number eighteen, and then we let the mercury sit for twenty minutes. After the mercury soaked our cells in a small petri dish for twenty minutes, we removed the mercury and discarded it properly into a waste container. Then continued with the same steps starting again from step twenty-one.

Imaging our control and our experimental used epi- fluorescence illumination on a fluorescence microscope. These methods can be seen on “ Morris (2011d)”. As for the materials they can be seen in the list labeled materials called “Viewing and imaging data materials” list. For both our control and experimental images we went through the process of (2011a,c) but captured images after changing the buffer in increments of one minute twice, so that two images were captured for the control and the experimental region.

Result:

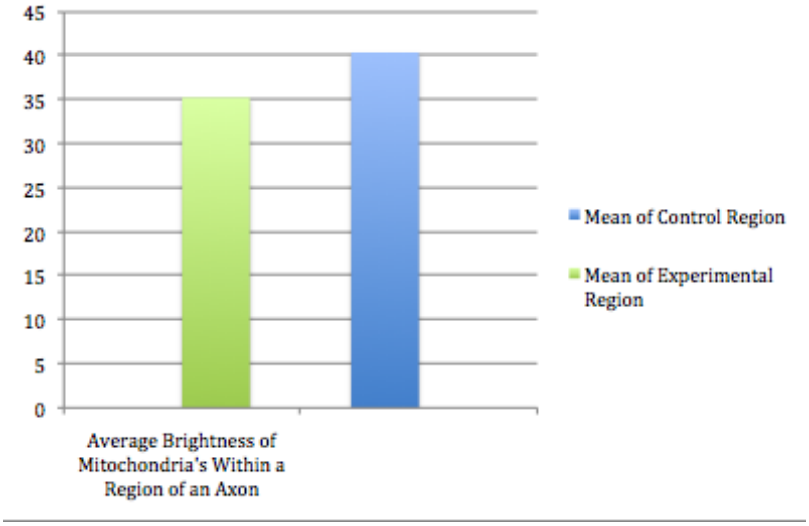
The quantifiable data appropriate to our results was to record the mitochondria brightness to show whether

mitochondria in the control or experimental are brighter and therefor have a higher charge than one another. The brighter the mitochondria are in one specific tested region of the axon the greater the charge of the mitochondria in that one tested region.

Image J software was used to square off and isolate the regions of the axons in a polygonal shape that included mitochondria, and calculated an average/mean which was then recorded as major data. The mean of the mitochondrial region was done by the Image J software, which was calculated by the LUT (look-up-table) that is superimposed on the pixel brightness histogram for an image. The procedure to adjust the Max and Min of the B&C window with the LUT can be seen on “Morris (2011e)”. This process was done for both an experimental and control region of the imaged axons with the most mitochondria visible.

A region in the axon of the image of the control and experimental with the most mitochondria’s visible was recorded. Our results conclude that the brightness of the mitochondria’s in the controlled axon region is brighter than the brightness of the mitochondria’s in the experimental axon region that was exposed to mercury. This Conclusion can also be observed in Figure 1.

Figure 1 Average Brightness of Mitochondris within a Region of an Axon



- Figure one shows an average/mean of the brightness of one sectioned off region of an axon that contains mitochondria (n=1 region of an axon that was measured in brightness). Brightness corresponds to charge, and the brightness was calculated in megapixels on Image J software that quantifies a total mean for each region tested. There is only one region in each of the control axon and only one experimental axonal region used to quantify this data.

Figure 2 A Control Axon Image

(Using R123 and washed in DMEM buffer)

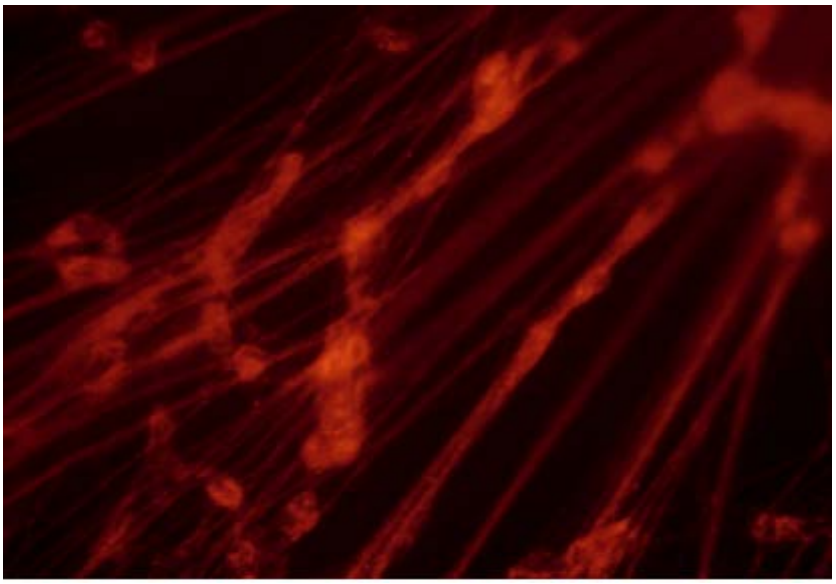
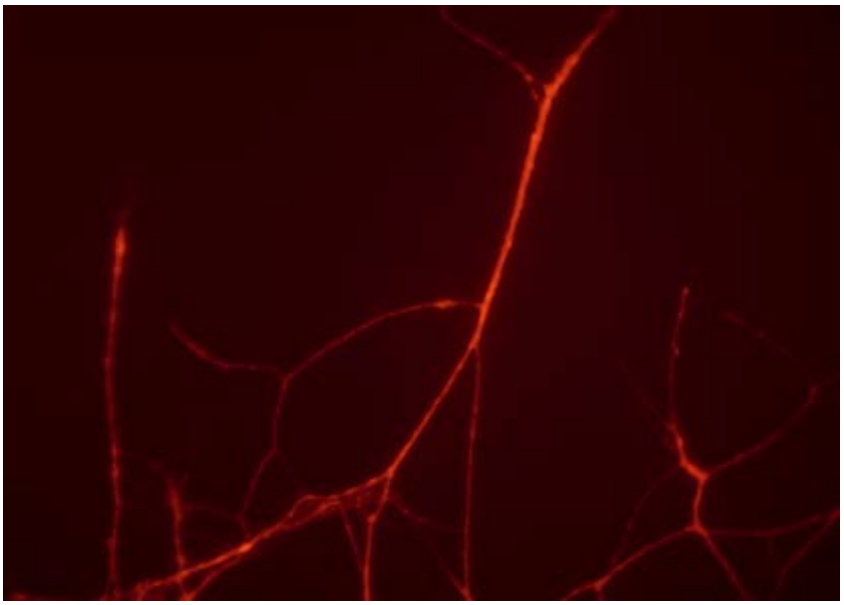


Figure 2 B Experimental Mercury Exposed Axons

(Using R123 and washed in Mercury then DMEM buffer)



Discussion & Conclusion:

My hypothesis throughout this experiment was that mitochondria in control growing axons would have a higher charge than mitochondria in mercury exposed retracting axons. Mitochondria in retracting axonal regions exposed to mercury have a lower charge than mitochondria in controlled growing axonal regions. According to my results my hypothesis was supported. My data showed that when in the control experiment mitochondria were highly charged. The experiment with mitochondria that were exposed to mercury was not as bright as the control axon regions. This suggests that mercury has an effect on mitochondrial charge when a cell is exposed to it for a period of time. As previously mentioned, there are other experiments done with mercury exposure to cells that demonstrates that mercury

disrupts membrane structures like the mitochondria (Leong, Syed et al. 2000). Therefore according to Leong, and Syed the mercury may disrupt the activities of the mitochondria as seen in this study through the brightness of the mitochondria. This is possible because mercury disrupts membrane structures in a cell (Daytonmedical 2011). This explains why the average brightness of mitochondria in the axons that were exposed to mercury was not as great as the control axonal region with no mercury exposure.

If this experiment were to be refined, I believe that the final outcome would support my hypothesis. My study reveals that mercury has an effect on cellular biology. To demonstrate my study in a more effective way, some future experiments would be to quantify the charge of mitochondria in multiple regions of an axon and test if mitochondrial brightness corresponds with either retracting axons to mercury or growth of controls

References Cited

- Ekizian, D. (2007). Mitochondrial disorder medical information. Retrieved from http://www.mitoaction.org/medical-information?gclid=CLqU_9ChtKwCFQM75QodUnlvGQ
- Garrecht, M., & Austin, D. W. (2011). The plausibility of a role for mercury in the etiology of autism: A cellular perspective. Toxicological and Environmental Chemistry, 93(6), 1251-1273. Retrieved from www.scopus.com
- Leong, Christopher C. W., Naweed I. Syed, and Fritz L. Lorscheider. "Retrograde Degeneration of Neurite Membrane Structural Integrity of Nerve Growth Cones following in Vitro Exposure to Mercury." *Neuroreport* 12.4 (2001): 733-37. Print.
- "Mercury." *Dayton Medical Therapy, Practices, Nutrition, Conditions Treated | Sunny Isles Beach, Florida*. Daytonmedical, 2011. Web. 29 Nov. 2011. <http://www.daytonmedical.com/mercury.php>.
- Morris, Robert L., and Peter J. Hollenbeck. "The Regulation of Bidirectional Mitochondrial Transport Is Coordinated with Axonal Outgrowth." *Journal of Cells Science* (1993): 917-27. Print.
- Morris, R.L. (2011a) Neurobiology Bio324 Primary Culture Of Chick Embryonic Peripheral Neurons 1: DISSECTION. Available at http://icuc.wheatonma.edu/bio324/2011/LabProjProc/1.htm
- Morris, R.L. (2011b) Neurobiology Bio324 Primary Culture Of Chick Embryonic Peripheral Neurons 2: OBSERVATION. Available at http://icuc.wheatonma.edu/bio324/2011/LabProjProc/2.htm.
- Morris, R.L. (2011c) Neurobiology Bio324 Primary Culture Of Chick Embryonic Peripheral Neurons 3: STAINING and OBSERVATION. Available at http://icuc.wheatonma.edu/bio324/2011/LabProjProc/3.htm

- Morris, R.L. (2011d) Neurobiology Bio 324, NBlab Primary Tiss Culture proc 2 Observn 2001c. doc. Fluorescent Microscopy.
- Morris, R.L. (2011e) Neurobiology Bio324, Primary Culture of Chick Embryonic Peripheral Neurons- Quantitation
- Sullivan, Jim. "About the CELLS Alive! Website." *Home of CELLS Alive!* Quill Graphics, May 1994. Web. 13 Oct. (2011). <http://www.cellsalive.com/aboutus.htm>.
- Voloboueva, L. A. and Giffard, R. G. (2011), Inflammation, mitochondria, and the inhibition of adult neurogenesis. Journal of Neuroscience Research, 89: 1989–1996. doi: 10.1002/jnr.22768