

# Mitochondria function in response to *in vitro* exposure to mercury

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## Introduction

Mitochondria are double membrane bound organelles that provide the energy a cell needs to move, divide, and contract (Henze & Martin, 2003). The inner membrane of mitochondria folds inward creating cristae (Paumard et al, 2002). These cristae are extremely important to the cell because it is the location where sugar bonds with oxygen to form ATP- the primary source of energy for the cell.

Mercury (Hg) is a naturally occurring metal and toxic that targets the central nervous system (Benoit, 2011). Several mechanisms have been proposed to explain how mercury can kill neurons. Some of these mechanisms are disruption of mitochondria function, direct affect on ion exchange in a neuron, and destruction of the structural framework of neurons (Chudler, 2011). There are reports that relate the toxic effects of mercury to loss of mitochondrial function such as such as inner membrane depolarization, and loss of oxidative phosphorylation (Southard and Nitisewojo, 1973).

This experiment focuses on the effect of mercury on mitochondrial ATP generative capacity. Mitochondria have been implicated as an important subcellular site of damage caused by Hg (Southard, 1973). In this study I examined mitochondrial activity, or mitochondria ATP generative capacity, in the axons of peripheral neurons of 10 day old chicken embryos (*Gallus gallus*). The respiratory chain in mitochondria is its ability to generate ATP by means of the electron transport chain and oxidative-phosphorylation system (DiMauro & Schon, 2003). I hypothesize that mercury causes a respiratory chain impairment in mitochondria and therefore impedes mitochondria's ability to produce ATP. To test this hypothesis I observed and quantified mitochondrial fluorescence using rhodamine- 123 before and after mercury exposure. Because rhodimine-123 is a lipophilic cation, it causes negatively charged structures to be stained brighter then the rest of the cell. The more negative the mitochondria were, the brighter they appeared and the more electron transport they underwent, therefore producing more ATP (Morris, 2011 Lab). My data reveal that mitochondria become less negative after mercury exposure, thus having less ATP-generating capacity.

## Materials and Methods

### Materials

Tissue culture media and supplements, rhodamine-123 (10mg/ml) and mercuric chloride (10  $\mu$ m in 0.5% HCl) were obtained and used for this experiment.

## **Cell Culture**

Sympathetic chain ganglia and dorsal root ganglia were dissected and cultured as whole or dissociated ganglia and grown as single cells on coverslips previously described (Morris, 2011a). Cells were grown for 18-26 hours in a humidified incubator in Liebovitz L-15 medium supplemented with .5% methyl cellulose, 2 mM glutamine, .6% glucose, 100U,  $\mu$ g/ml pen/strep, 10% fetal bovine serum, and 50 ng/ml nerve growth factor. Coverslips used for the culture were treated with polylysine and laminin before ganglia was plated. A flow chamber was prepared, and the coverslips were stained with rhodamine-123 using a procedure previously described (Morris, 2011c).

## **Control**

After dissection and plating, a flow chamber was prepared, and the coverslips were stained for fluorescent vital staining as previously described (Morris, 2011c). Four phase pictures and four fluorescent pictures were taken then growth medium was flown through the chamber. Eight more pictures (4 phase, 4 fluorescent) were taken immediately after the flow took place to ensure that the cells were not disrupted from it.

## **Experimental**

The same procedure as the control data was followed for the experimental data, except instead of flowing growth medium through the chamber, mercury was flown through chamber. Eight pictures were taken before and flow took place. After flowing mercury through the chamber, the slide was allowed to sit for 20 minutes. The mercury was then washed out but flowing growth medium through the chamber. Eight more pictures were taken.

All images were taken using a Nikon Eclipse E200 with phase options, and objective lenses. The samples were viewed at 40x. They were imaged through a Spot Insight Firewire 2 Megasample at 1.0x C-mount. The images were observed with Spot Software Version 4.6.1.26 on a IMAC computer running MAC OS X Version 10.5.8 in the ICUC at Wheaton

## **Data Analysis**

To determine the brightness of mitochondria I used ImageJ 1.45s on a Toshiba Satellite L745 running Windows 7 Home Premium. Because I needed to account for retrograde degeneration of neuritis following mercury (Leong et al, 2000) I selected sections that were 200 pixels away from the growth cone to measure brightness. I quantified a ratio of mitochondria brightness to the background by measuring an area with mitochondria and an area of the background using ImageJ.

## **Results**

To account for any excess rhodamine-123 that may not have washed off the coverslip completely, I used a ratio of mitochondria brightness to background for both before and after mercury images. Figure 1a, before mercury exposure shows a robust amount of mitochondria towards the end of the growth cone, and had a mitochondria brightness to background ratio of 2.52 which was the second highest ratio in the data. The highest mitochondria brightness to background ratio was 3.78 in an image post mercury exposure. Figure 1b is an image of a neuron after mercury exposure. Not only is it less bright than the pre-mercury exposure picture but it also shows considerable retrograde degeneration of the neurite which is why measurements were taken 200 pixels away from the growth cone. The mitochondria brightness to background ratio was 2.27. The average mitochondria to background ratio was 2.52 for images pre-mercury exposure and 2.26 for images post-mercury exposure.

Figure 1a.

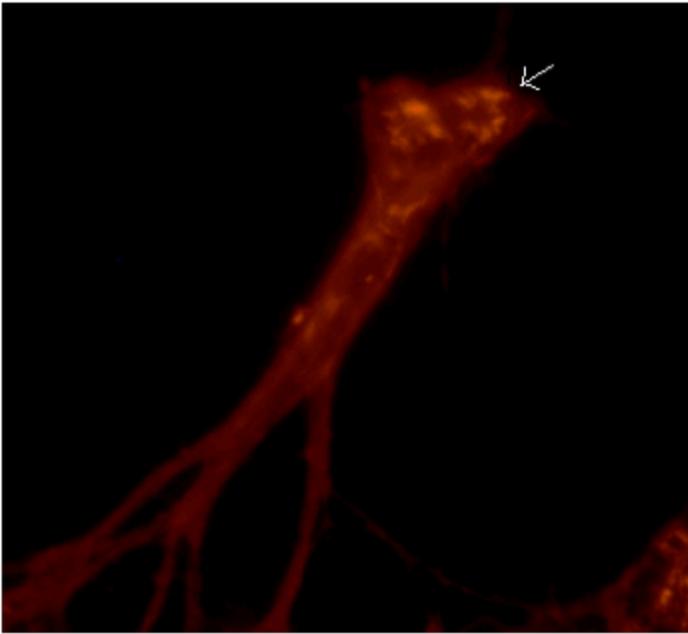


Figure 1b.

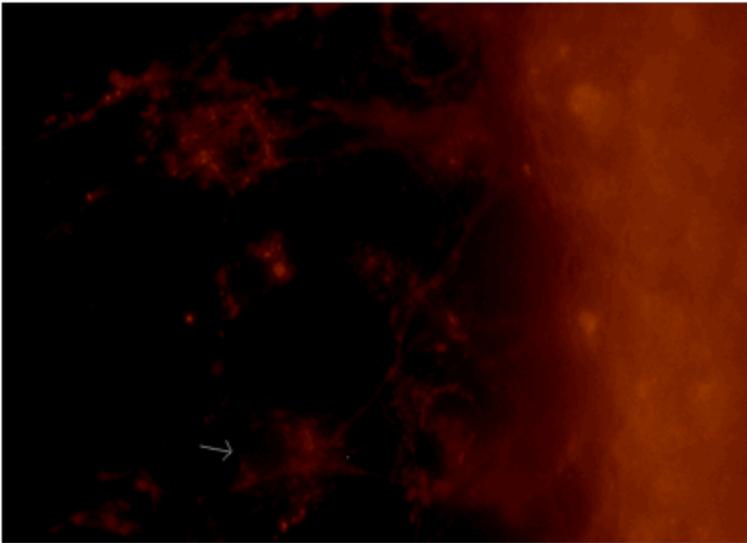


Figure 1. A live ganglia with rhodamine-123 fluorescent vital dye. (A) The ganglia pre-mercury exposure. Notice the abundance of mitochondria toward the growth cone. (B) The ganglia post mercury exposure. Notice the decrease in fluorescence and the retrograde degeneration of the neurites. Arrows indicate location of a growth cone.

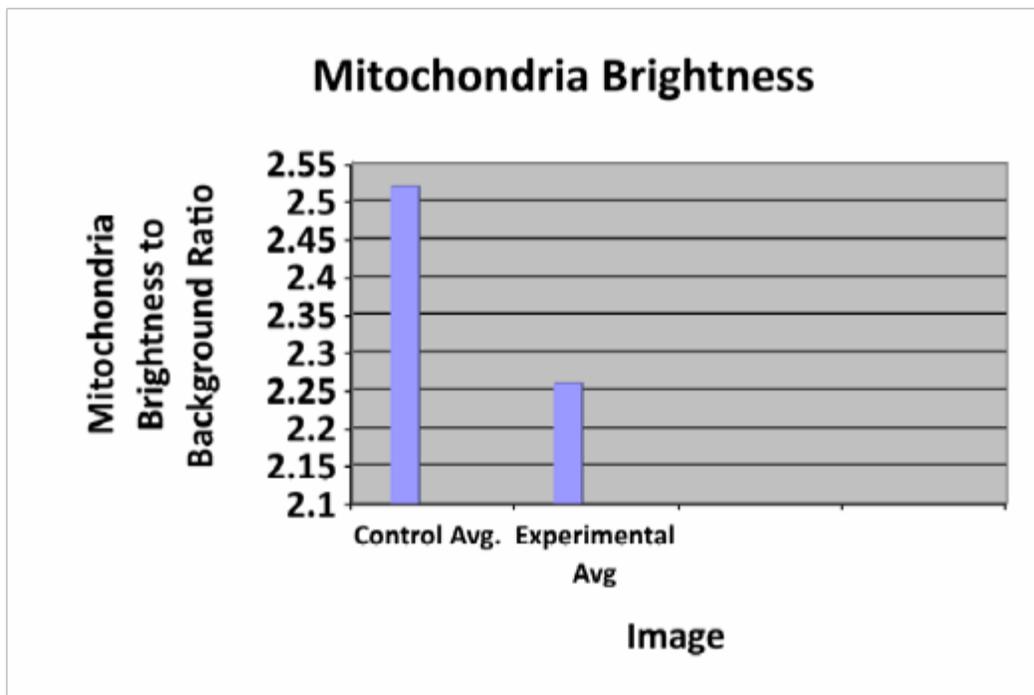


Figure 2. The average ratio of mitochondria brightness to background ratio for controls and experimental images. Notice the higher ratio of controls, pre-mercury exposure. Data were generated from on 8 growth cones from 2 cells during 2 trials, n=8.

## Discussion

I found that mitochondria pre-mercury exposure had greater fluorescence than mitochondria that had been exposed to mercury. There was only one case in which exposed mitochondria had a greater mitochondria fluorescence than non-exposed mitochondria. Morris and Hollenbeck (1993) found that in growing neurons, the growth cone forms a focus of ATP consumption that requires the positioning of mitochondria near the growth cone. Leong and colleagues (2000) found that mercury exposure causes retrograde degeneration of neurites. Because of these findings I believe that the retrograde degeneration of the neurites I observed in my experiment, also caused mitochondria to move retrogradely down the axon. This may have produced a cluster of mitochondria in the measured area which would cause that area to appear brighter because of the abundance of mitochondria. The measurement I did 200 pixels away from the growth cone may not have been far enough from the growth cone to account for retrograde degeneration in this particular neurite.

If this experiment was repeated multiple times, and generated the same results, it would provide much evidence for the effects of mercury on mitochondria ATP generative capacity. Also the ideal control would have been allowed to sit for 20 minutes after the growth medium was flown through because the experimental was allowed to sit for 20 minutes. Future experiments could measure mitochondria brightness further away from the growth cone since data from this experiment may still have not completely accounted for retrograde degeneration of neurites.

This experiment indicates that mitochondria are a site of damage for mercury, and the mercury affects ATP

generative abilities in mitochondria. This may be by some disruption in the electron transport chain in mitochondria (DiMauro & Schon, 2003).

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