# Nerve growth cone area decreases after mercury exposure

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## Introduction

Coal-fired power plants are the source of 50-75% of the atmospheric mercury pollution in the United States (Kuntz, Ricco, Hill, & Anderko, 2009). Atmospheric mercury is converted into ionic mercury accumulating in the ocean by rainfall (Kuntz et al., 2009; Holmes, James, Levy, 2009). Plankton absorbs this rainfall containing and methylmercury bioaccumulates up the food chain to humans, each step increasing the concentration 4-5 fold (Kuntz et al., 2009). Direct exposure to elemental mercury also occurs via dental amalgams and inhalation of vapor from Hg spills (Kuntz et al., 2009)

This is dangerous to humans because mercury is neurotoxin that can cross the blood-brain barrier and the placenta (Holmes et al., 2009). The fetal brain, containing growing neurons, is extremely susceptible to mercury-induced damage (Holmes et al., 2009). Low-dose mercury exposure to a fetus is associated with developmental delays, learning disabilities, and possibly behavioral problems (Kuntz et al., 2009; Holmes et al., 2009).

At the tip of developing neurons, growth cones are responsible for extension, and finding a path for the axon (Leong et al., 2001). Their cytoskeletons are comprised of actin and tubulin (Leong et al., 2001). Research has demonstrated that mercury causes microtubule disassembly in tubulin (Falconer, Vaillant, Reuhl, Laferriere, & Brown, 1994). Capo, Alonso, Sevil , and Frejo (1994) have also demonstrated that mercury can cause axonal and nerve degeneration. Leong et al. (2001) have supported the hypothesis that mercury markedly disrupts membrane structure and linear growth rates of neurites in growth cones. Finally, Pamphlett and Png (1998) found that when neurons are exposed to mercury, this causes a reduction in the size of axons.

This present study tests the hypothesis that mercury causes a reduction in the area of nerve growth cones over time after mercury exposure. This study treated embryonic *Gallus gallus* (a model for humans) sympathetic neurons with mercury solutions and measured the area of growth cones in a control trial and experimental trial.

## Materials and Methods

Materials

Materials used for this experiment were dissection and culture tools, DMEM, growth medium, live *Gallus gallus*

embryos, and mercury. Equipment used was a phase microscope, and still-frame imaging. Imaging was done at the Wheaton College ICUC using a Nikon EFD-3 with Sony DFW-x700 digital interface on Mac OS x Version 10.5.8 and data was analyzed using ImageJ software.

Methods

First primary culture of chick embryonic peripheral neurons of 10-day old chicken embryos were completed to create 2 cover slips containing F+ growth medium using 1/2 - 2/3 DRG per cover slip (Morris, 2011a). Cover slips were incubated at 37° Celsius for from 4 hours until overnight (Morris, 2011a). See Morris (2011a) for exact procedure. Then, flow chambers were created for each slide (Morris, 2011b). See Morris (2011b) for exact procedure on how to create, focus on, and image the slides. One growth cone for the control slide was focused on using the 40x objective. The same was done for the experimental slide. Using video-enhanced phase microscopy, initial images of each slide were taken. All images were taken in Neurobiology 324 lab in collaboration with DeNormandie (2011).

*Control*

Next a buffer exchange took place by adding a small pipette full of growth medium to the side of the cover slip on the control slide, collecting excess with a kimwipe on the other side. See Morris (2011c) for exact procedure. The same growth cone from the initial image was focused on and images were taken at 4 minutes, 8 minutes, 12 minutes, 16 minutes, and 20 minutes after the buffer exchange. One trial was done. A control trial was used inducing growth medium to ensure that any reduction in area was due to the mercury and not any outside environmental factors unrecognized.

*Experimental*

Next a small pipette full of 0.19mL of 100nM $HgCl_2$ was added to the side of the cover slip on the experimental slide, collecting the excess with a kimwipe on the other side. This exposure lasted 20 minutes. Next a buffer exchange took place by adding a small pipette full of growth medium to the side of the cover slip, collecting excess with a kimwipe on the other side. See Morris (2011c) for the exact procedure. The same growth cone from the initial image was focused on and images were taken at 4 minutes, 8 minutes, 12 minutes, 16 minutes, and 20 minutes after the buffer exchange. One trial was done.

Data Analysis

Areas of the two growth cones were calculated in pixels for all 12 images using ImageJ software, using a fixed point for each slide as the start of the area. Contrast was set to 48 minimum and 196 maximum for each image. The axon, lamellipodia, and filopodia were all included in the area. Next, the percentage of growth cone area was calculated for times 4, 8, 12, 16, and 20 for each trial using the initial image of each trial as the whole area. For example, the

percentage at 4 minutes was calculated by putting the area at 4 minutes over the initial (whole) area and multiplying this result by 100. This was done for each time point in both the control and experimental trials.

# Results

Data was collected for both control and experimental trials. Figure 1 depicts the nerve growth cone used for the control trial. The filopodium above the area that was measured was static the entire experiment. The point where this filopodium begins and perpendicular across the axon was used as the start point to measure the area for all images in the control trial. This figure is significant because it shows that the buffer exchange did not disrupt the integrity of the growth cone.

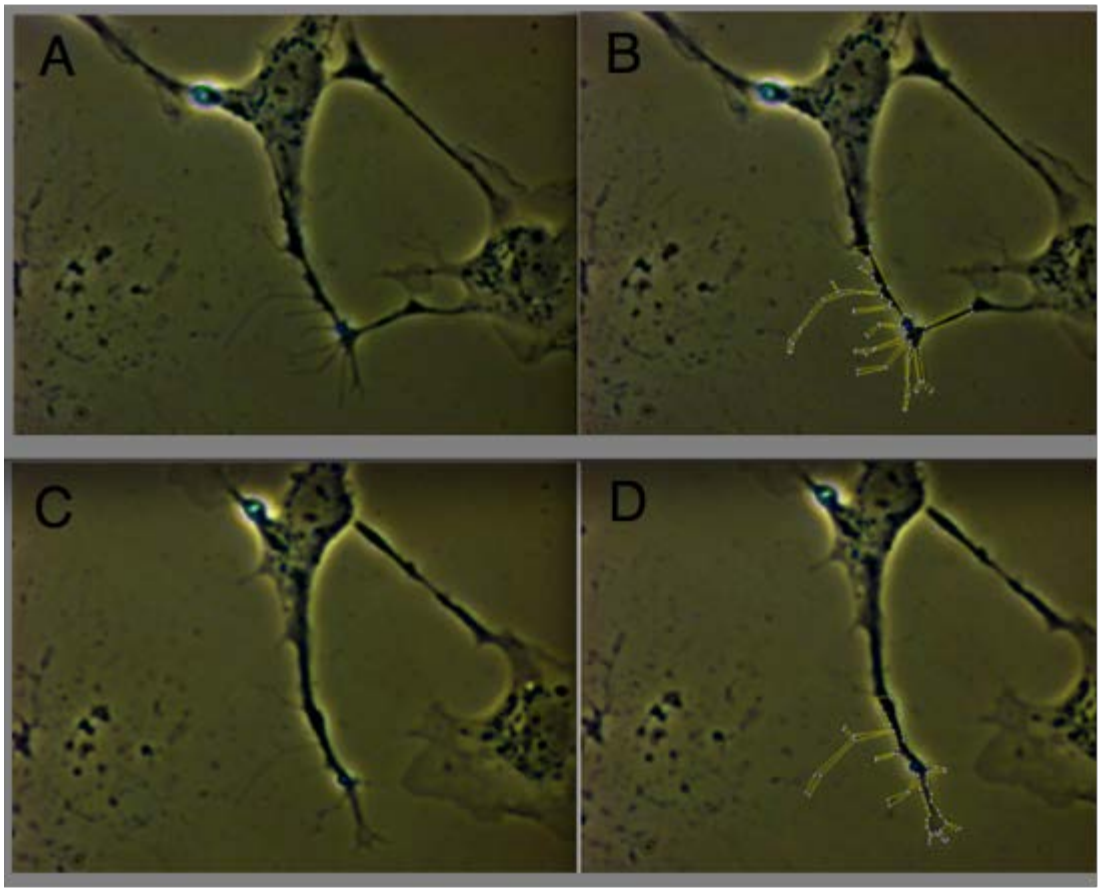


**Fig. 1**. Control images of nerve growth cone, initial image, and image at 20 minutes, n=1. This figure shows the growth cone imaged in the control trial. Part A is the initial image (Image 1 in Figure 3) of the growth cone before any treatment. Part B is the same image as part A with the area outlined using ImageJ. Part C is the image 20 minutes after the buffer exchange (Image 6 in Figure 3). Part D is the same image as part C with the area outlined using ImageJ. Notice the area of the growth cone in Part B and D are similar.

Figure 2 depicts the nerve growth cone used for the experimental trial. The filopodium below the area that was measured was also static the entire experiment. The point where this filopodium begins and perpendicular across the axon was used as the starting point to measure the area for all images in the experimental trial.

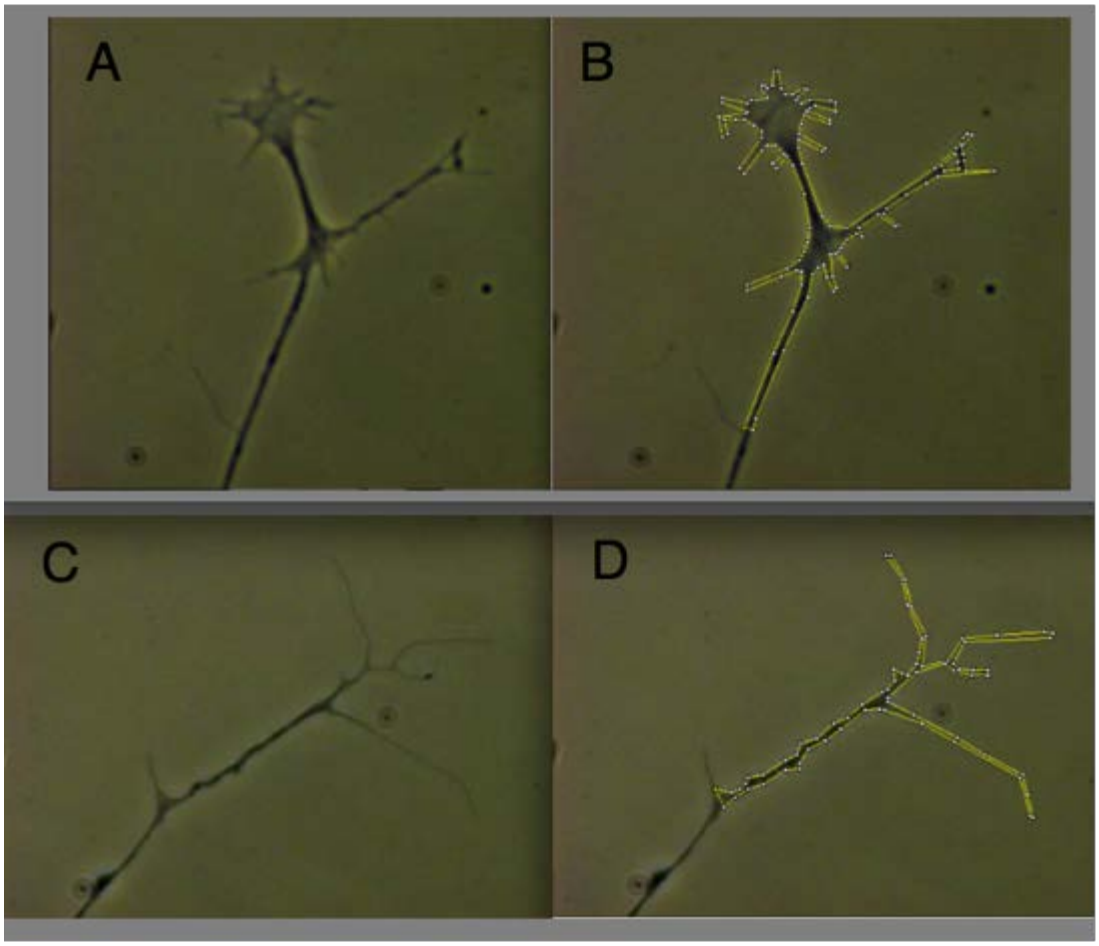


**Fig. 2**. Experimental images of nerve growth cone; initial image, and image at 20 minutes, n=1. This figure shows the growth cone imaged in the experimental trial. Part A is the initial image (Image 1 in Figure 3) of the growth cone before treatment. Part B is the same as part A with the area outlined using ImageJ. Part C is the image 20 minutes after the buffer exchange (Image 6 in Figure 3). Part D is the same image as part C with the area outlined using ImageJ. Notice the area of the growth cone in Part D appears to be less than the area of the growth cone in part B.

From these images, the areas were calculated using the measure function in ImageJ. Figure 3 shows the percent of initial growth cone size for the times measured, with the initial growth cone area being 100%. The initial measurement was taken before the addition of mercury, and images of the minute time points were taken after the exposure and washout of mercury. For the control trials, after the initial image, the percents fluctuated over time, but remained relatively constant. The experimental trial, however, showed a decrease in percentage over time. 4 minutes after the removal of mercury the percentage remained high. However, over the 20-minute interval, this area gradually decreased with the lowest area seen at 20 minutes. Overall, the growth cone after a 20 minute exposure of 100mN $HgCl_2$ in the experimental trial showed a significant decrease in area in comparison to the area of the growth cone in the control trial.

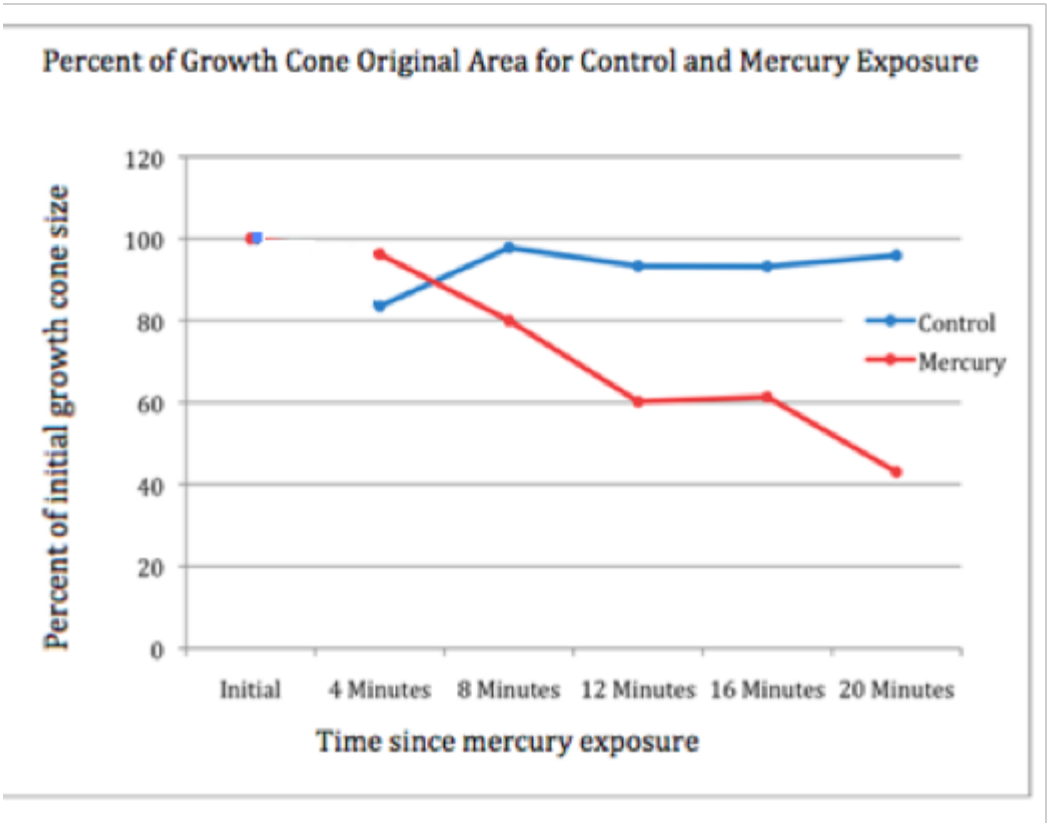


**Fig 3.** Graph of percent growth cone original area for control and mercury exposure trials. This figure shows how the area of the growth cones for both trials changed over time. The initial image was the very first image taken of the growth cones. The minutes stand for the amount of time that had passed after the mercury exposure had ended (meaning after washout). Notice the control percentages remain high, while the experimental percentages decline over time.

# Discussion

This present study supports the hypothesis that mercury causes a reduction in the area of nerve growth cones over time after exposure to mercury. Results show that the area of the mercury treated growth cone reduced to 43.0% of its original area 20 minutes after exposure. Area for the control remained much higher than the experimental, suggesting that mercury caused this reduction. Area reduction suggests that the nerve growth cone degenerated over time. Degeneration causes disruptions in brain development. If the growth cone cannot extend and find connections, then the brain cannot fully develop (Holmes et al., 2009), leading to developmental delays (Kuntz et al., 2009).

If this experiment were to be repeated a thousand times over with multiple trials for each experiment, generating the same results, then there would be much stronger evidence supporting that mercury has a negative effect on nerve growth cones. Mercury is a neurotoxin (Holmes et al., 2009), so from a cellular standpoint one would say that the cytoskeleton integrity of the nerve growth cone is compromised. This suggests that the cytoskeleton filaments, made of actin and tubulin, are depolymerizing due to the effects of mercury. Depolymerization is a removal of subunits at polymer ends (Falconer et al., 1994), thus causing a decrease in area. This depolymerization can cause the normal functions of growth cones to be compromised.

One source of error in this experiment is that surface area is not always an accurate measurement of size because thickness is not considered. Lamellipodia are more spread out than the filopodia, covering more surface area but this does not necessarily mean there are differences in the amount of actin and tubulin. To refine this experiment the thickness of the growth cone must also be measured for more accurate results. A second source of error is that the experimental trial received a mercury exposure lasting 20 minutes, and an observation lasting 20 minutes, while the control trial only received the observation time. To refine this experiment one must expose the control to a 20 minute buffer solution and then begin the observation.

Future experiments could look at longer and shorter time exposures to mercury to determine if there is a safe length of time that one could be exposed to mercury. They could also change the concentration of mercury for a better overall picture.

# References


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