Mitochondrial Abundance In Glia After Exposure To Methylmercury

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Introduction:

We studied net abundance of mitochondrial activity in the glial cells of chicken neurons after exposure to the toxin methylmercury. Our hypothesis was that glia exposed to methylmercury would have a significant reduction in their abundance, due to negative effects of the toxin.

Methylmercury (CH_3Hg or MeHg) is an industrial pollutant of the environment that primarily affects the nervous system. Symptoms of methylmercury poisoning include paraesthesia, ataxia, dysarthria, and hearing loss. Humans and animals convert methylmercury to inorganic mercury in their bodies. (IPCS 1990) There mercury acts as a neurotoxin by inhibiting the action of enzymes containing selenium, although much of mercury's activity in the body is not well understood. (Carvalho et al., 2008) This neurotoxin has been especially problematic in communities affected on a large scale by exposure, such as the thousands of people poisoned by the disposal of methylmercury-containing waste in Minamata Bay in Japan during the 1950s and 1960s. In that case, patients continued to be affected by the toxin decades after it ceased to be released in the bay. Several large-scale poisonings have happened around the world since. (Harada, 1995).

Recent studies have focused on the effects of methylmercury and its derivatives on the cells of the nervous system. Methylmercury is a known cause of mitochondrial apoptosis (cell death) and malfunction in neural cells. (Toimela & Tähti, 2004) This eventually results in whole-cell apoptosis of the neural cells the mitochondria are in. (Ceccatelli et al., 2010) However, to our knowledge no study has been published regarding the effects of methylmercury on glial mitochondria in particular. Our hypothesis, based the studies of Toimela & Tähti and Ceccatelli, is that the methylmercury will reduce the mitochondrial abundance in glia exposed to it compared to glia not exposed to the toxin.

Glia are also desirable for observation of mitochondrial abundance in vitro because of their properties that differ from neuron cells as well. In neurons, since protein particles and organelles are actively transported along the axon and

dendrites and pass through the cell body, it is likely that a large amount of methylmercury will pass through the cell body as well unintentionally. The less active glial cell, in contrast, can keep mitochondrial survival higher because there are fewer demands on the cell. (Kandel et al. 2013)

We used neural cells taken from fetal chickens (*Gallus gallus*) for the purposes of this study. Fetal chicken cells have a number of advantages: they can be replaced easily and quickly, their close relationship to humans as terrestrial vertebrate animals, and their rapid growth in medium. Chickens also have similar physiological reactions to methylmercury exposure to humans, as do all animals. (IPCS, 1990)

My collaborator was Hazie Crespo, who worked on the same hypothesis with the same experiment. We are working on the same problem to increase the amount of data we can collect. We also tried to reduce individual skews and unforeseen variables that minor differentiations in our analytical procedures may unintentionally create by ensuring that the experiment is reproducible by more than one person. This ensured that our experiments are not outliers or being influenced by any unknowns.

In this study, we observed the change in mitochondrial abundance between a fetal chick cell culture exposed to methylmercury and a control culture via measuring the background-adjusted brightness of dyed mitochondria.

Materials and Methods:

Only materials directly used in this experiment will be considered here. As stated above, neural cells from fetal chicks were cultured for observation. The cells came from dorsal root ganglions of the fetus removed from the egg and dissected. Each culture originated from one fetus, with multiple ganglions of each fetus creating each culture.

There were two media used in this experiment: a growth medium and Dulbecco’s modified Eagle's medium (DMEM). The growth medium was specially made for this experiment for optimal neural cell growth. Its makeup is described in Morris (2014a). It consists of Leibovitz L-15 medium plus 10% fetal aclf serum, 0.6% glucose, 2 mM L-glutamine, 100 µg/ml streptomycin, 100 U/ml ppenicillin, and 10-50 ng/ml nerve growth factor (NGF). The DMEM used was the standard stock medium. However, a version of the DMEM with 40 nM methylmercury added to DMEM in a 1:100 dilution was the delivery method for methylmercury to the cell cultures. MitoTracker is also used in the experiment. MitoTracker is a chemically active fluorescent dye that becomes permanently bound to the mitochondria and signifies their activity by glowing brighter with increased membrane potential. (Chazotte 2011)

Microscope slides, coverslips, and coverslips chips were used to make the observation chamber. VALAP wax formed the walls of the chamber. The microscope was a Nikon E400 microscope using a 40x lens. Images from the microscope were photographed with the SPOT imaging software running on the “Capricorn” iMac G4 attached to the microscope. For the analysis of the results, the free image editing software Image J 1.40 was used for determining the brightness of the mitochondria.

The method of the extraction of the dorsal root ganglions is from Morris (2014a). It is not included here as it is not pertinent to this experiment in particular. Most of the procedure that follows is adapted from Morris (2014b). However, there are some sections of the method that are specific to this experiment. If not mentioned below, the procedure is identical to the vital-stain fluorescence microscopy procedure there. For this experiment, the procedure was done twice: once for a control medium, and once for an experimental medium.

Preparation of the chick neural cultures began by removing the growth medium from the Petri dishes containing the cultures in medium. DMEM containing methylmercury (MeHg) was added to the culture. In the control, DMEM without MeHg was used instead.

The petri dish was then incubated for 10 minutes. After removal from the incubator, the DMEM (in both cultures) was washed out three times with fresh DMEM. Finally, new DMEM was added, as well as the MitoTracker. Then the petri dish was immediately incubated again for 10 minutes. After removal from the incubator, the DMEM and MitoTracker (in both cultures) was washed out three times with fresh DMEM.

The coverslip was pried off the petri dish and put on a microscope slide with coverslip chips. VALAP was added to all four sides to make an observation chamber. The observation chamber was then taken to the ICUC where glia were photographed at 40x magnification on the Capricorn computer. All glia were viewed in true-color before the microscope switched to fluorescent light to view them.

One fluorescent image was taken of glia in the control group and one fluorescent image was taken of glia in the experimental group. Only the highest-quality images of unique glia are reported here. This particular subset of our data was used because it provided both the physically largest and visually concentrations glia cells we could find in our cultures. Although a larger n value of glia would be beneficial, these are still the highest quality images we created from the culture. Other photographs were taken of various glia but are not reported here due to the glia not being large or clear enough. Difference in the size of the glia does not bias the measurement as the average brightness throughout the entire mass of glial cells is measured, as explained below.

ImageJ 1.40 software was used to measure the value histograms of the images taken. Value is a technical measure of the brightness in each individual pixel of an image, measured in 8-bit color space from 0 to 255 in ImageJ. A histogram is a graph showing the pixel values over the image or a section of the image. (Rasband, 1997) All values were measured in polygonal figures enclosing the glia, labeled in the figures showing the photographs. The area enclosed by the polygon was determined by the edge of the background values, where the edges of the glia were located.

Different images were taken at different exposure times to make for the clearest possible image, but this had the effect of influencing the brightness of the image. Therefore the photographs were altered in ImageJ to give their backgrounds the same value. The photograph with the darkest background, the experimental photograph, was used as a reference, and the other image, the control photograph was darkened until its background matched the reference. The background of the control photograph had an average value of 31.5, while the background of the experimental photograph had an average value of 14.9. 31.5 divided by 14.9 gave us 2.1, so the value of every pixel of the image in the control photograph was divided by 2.1 to remove the effect of the differing exposure time. This also had the effect of lowering the brightness of the images as a whole, including the mitochondria. However this produced more accurate images that simulated the effect of identical exposure times.

Results:

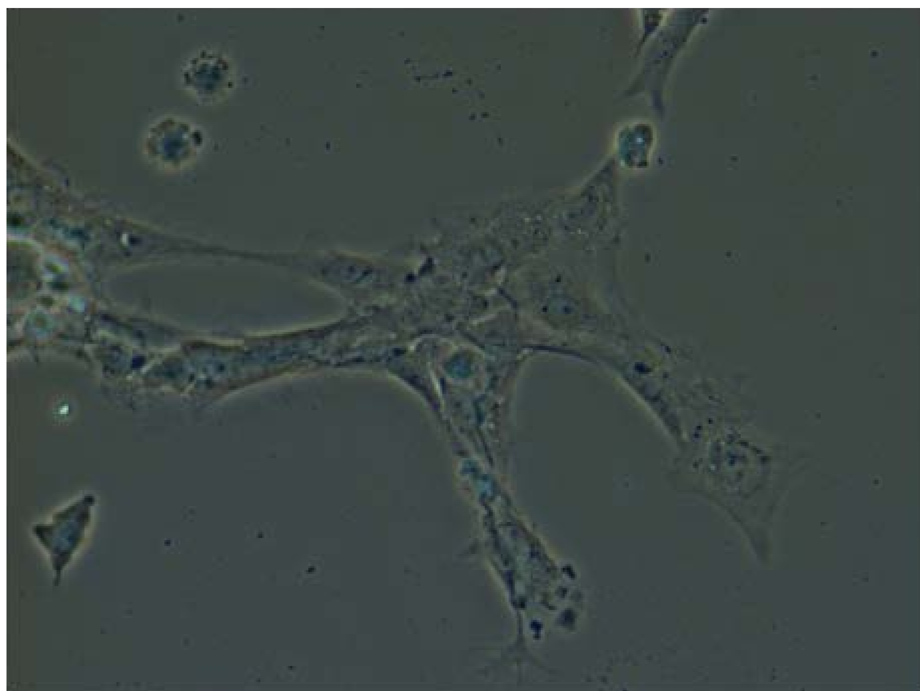

Fig. 1 (True-color view of control glia before fluorescent microscopy. Image taken with collaborator Hazie Crespo.)

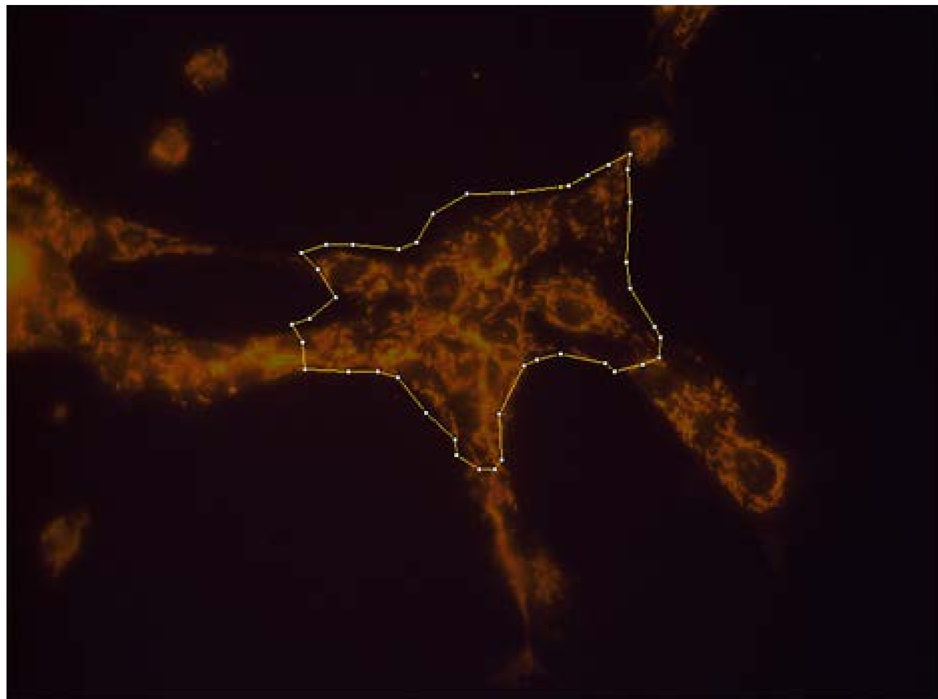

Fig. 2 (Figure of view of control glia after fluorescent microscopy, taken by Hazie Crespo and I, and its corresponding histogram.)

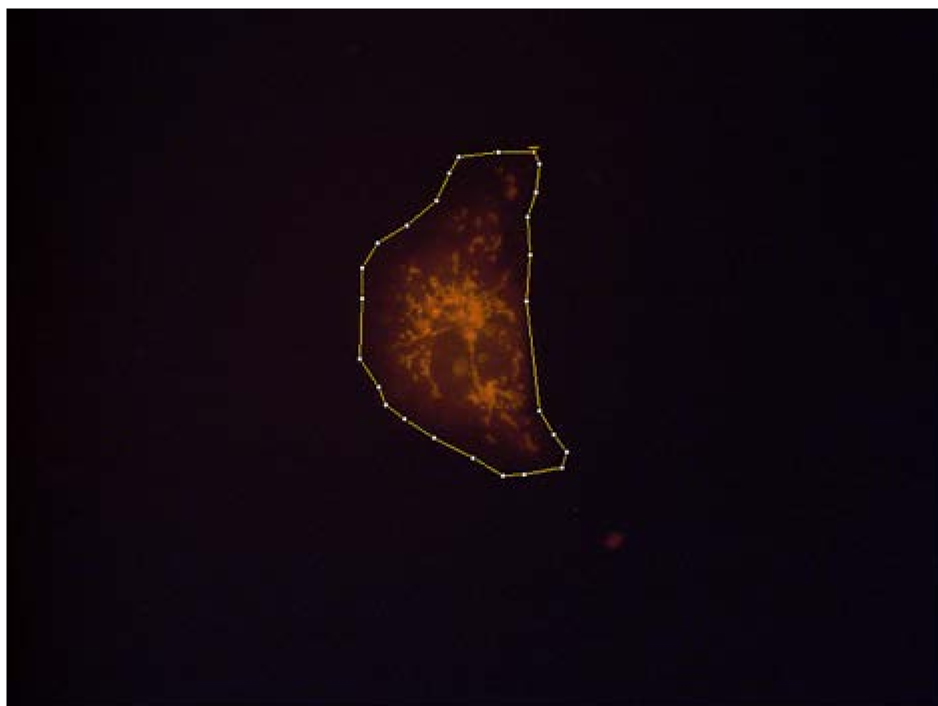

Fig. 3 (Figure of view of experimental glia after fluorescent microscopy. Image taken with collaborator Hazie Crespo.)

	Area	Mean	Min	Max
1	170218	34.491	10	71
2	125490	33.134	7	115

Table 1 (Chart of comparative results from histograms of the two images. “1” is data for the photograph of the control glia, while “2” is data for the photograph of the experimental glia. The mean brightness of the two glia are nearly identical.)

Discussion:

The methylmercury-exposed glia did not have any significant reduction in mitochondrial abundance when compared to the control group. After adjustment of the background value (brightness) as explained above, the average values of the glia in both pictures were nearly identical. These observations do not confirm our hypothesis, as our hypothesis predicted that there would be a measurable difference in mitochondrial abundance between the two cultures, with the experimental group having less abundant mitochondria.

A major weakness with this experiment is that only one culture each of the control group and experiment group was analyzed, and that only one fluorescent photograph taken of each was suitable for this experiment. This prevents us from drawing any final conclusions from this data in particular. If there were more control and experimental groups, the existence or nonexistence of a gap between control and experimental mitochondrial activity would have more data in support of it. However the experiment as it stands cannot reliably detect any effect of methylmercury exposure on the mitochondrial abundance chick glia. To refine this experiment, we would use more cultures in both groups, and take more photos in aggregate.

Future experiments could measure comparative abundance of mitochondria at different methylmercury levels. They could also measure or comparative abundance of mitochondria in glia and neurons exposed to the same amount of methylmercury.. It would be expected that the abundance of mitochondria is more reduced from the control level percentage-wise in neural cell bodies than glial cells, due to the higher reliance of neurons on mitochondria. The reduction may be more pronounced at higher levels of methylmercury, but the neural cells should be consistently more reduced in mitochondrial abundance than the glial cells.

Outside of comparative studies, an innovative line of experiment could center on the addition of selenium to the culture. Selenium has long been known to counteract methylmercury toxicity. (Stoewsand, Bache, and Lisk, 1972) This counteraction extends to fetal humans, who are most at risk to methylmercury to begin with. (Choi et al., 2008) Based on these studies, it is probable that mitochondrial abundance would be higher in glia exposed to both methylmercury and selenium than glia exposed to only methylmercury. However, few studies have investigated the interactions of the two elements in vitro as of this writing.

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