

# The Effect of Methyl Mercury on the Axon-Glia Interaction

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## Introduction:

Glial cell has an important role in the nerve system. There are three types of glial cells, Schwann cells, oligodendrocytes, and astrocytes. Many voltage-sensitive ion channels and neurotransmitter receptors of neurons are found in glia, and glia plays an important role in indirectly sensing the level of neuronal activity. They wrap layer of myelin membrane around the axon could insulate peripheral nervous system (PNS) and central nervous system (CNS) for impulse conduction (Fields & Stevens-Graham, 2002). Furthermore, Fields and Stevens-Graham (2002) also find that in the PNS, the axon inhibits the proliferation of Schwann cells. The axon-glia signaling is found to be extracellular ATP released from the non-synaptic region. Glial cells also are used as a “guide posts” to form some synaptic connections by the extended axons and dendrites. When the axons get injured, the myelin protein would hinder the axon regeneration in the CNS (Fields & Stevens-Graham, 2002). Glial cell also has the function of structural boundary to other tissues, formation and maintenance of central nervous system myelin (Aldskoquius & Kozlova, 1998). When glial cells and axons interact with each other, the functions of glial cells, such as maintaining ionic and chemical environment, structural support, storing nutrients to proved energy, providing immune system functions, can then be carried out (Meyer & Quenzer, 2013, p. 50).

Mercury, also known as the chemical symbol Hg, is a toxic metal in liquid form. It has shown that the toxicity of mercury could cause cell death of oligodendroglial cells. The toxicity of mercury could also lead to chronic fatigue, depression, poor memory, and a series of impairments in nervous system (Stem Cell Therapies, n.d.). Research done by Gradjean (as cited in Shea, 2004) also indicated that mercury would slow the brain’s response to sound, the signals was delayed under the effect of mercury along the transmission line from ear to brain through auditory nerves. (Shea, 2004) Meanwhile, the major source of mercury contamination in the environment comes from methyl mercury. The industrial processes produce wastewater, which contains a great amount of methyl mercury that could contaminate the fish in the river and sea. Human who consume these polluted fish would get a higher level of infection by methyl mercury (Akagi et al., 1995). Crespo-López and her colleagues (2007) also demonstrated that exposing methyl mercury to the nerve cells produced genotoxic effects in the CNS. Methyl mercury has been treated as a potent neurotoxic, and has a very obvious effect on disrupting the membrane structural integrity of neurites and the growth of neurons. (Leong; Syed and Lorscheider, 2000)

In this research, the effect of methyl mercury on axon-glia interaction has been tested because of the important contribution of axon-glia interaction to our CNS and how methyl mercury could harm these connections. My collaborative group (Molly and Tracy) and I used an optical microscopy technique to observe the ability of methyl mercury affect the axon-glia interaction in domestic chicken (*Gallus gallus*) neurite by using normally growing cell culture as a control group and pre-treated methyl mercury cell culture as an experimental group. The methyl mercury slows down or stops the growth of nerve cells and glial cells, and the growth medium that contains neuron-growing factors promotes nerve cells to grow. Due to the toxicity of mercury and its negative impacts on nervous system, the

presence of methyl mercury in the experimental group would impair the interaction between axon and glia. Based on these supporting backgrounds, I hypothesized that axon-glia interactions would decrease under the effect of methyl mercury than that in the normal growing environment. The cells would be observed under a phase microscopy and data would be collected through taking still images of the cell cultures.

## **Material and Methods:**

### Dissection and poly-lysine treatment

Domestic chicken (*G. gallus*) DRG and sympathetic chain were acquired by dissecting 10-day chicken embryo. Coverslips that were used in the experiment were cleaned and coated with poly-lysine by following the coverslip cleaning and coverslip treatment part1 and part 2 in the “Primary Culture of Chicken Embryonic Peripheral Neurons 1: Dissection.” (Morris, 2014) The detailed procedures of dissection were demonstrated in the “Primary Culture of Chicken Embryonic Peripheral Neurons 1: Dissection, dissection part1 and part2”. (Morris, 2014) The cells were treated with trypsin and suspended in order to dissociate into single cells. Specific steps were indicated in the “Primary Culture of Chicken Embryonic Peripheral Neurons 1: Dissection, dissociation of ganglia”. (Morris, 2014a)

### MeHg treatment and plating cell cultures

The dissociated DRG cells were plated out in growth medium and incubated at 37°C overnight; detailed procedure was indicated in the “plating out” part (Morris, 2014a). The cells cultures with normal treatment that was described in Morris lab guide line--Plating Out was treated as the control group (Morris, 2014a). According to previous research done by Leong, Syed and Lorscheider (2000) and our own calculations, a dose of 40nM concentrated methyl mercury (MeHg) in the DMEM solution was pre-added into the neuron cell culture. Then the mixture of cells and MeHg DMEM was added into growth medium for incubation overnight. MeHg was presented and remained in the cell culture for entire time of cell growing. And, these MeHg pre-treated cell cultures were the experimental groups.

### Measurement and data analysis

The control group and experimental group cell cultures were made into chip chambers followed by the procedure in part B in “Primary Culture of Chick Embryonic Peripheral Neurons 2: Observation of Live Unlabeled Cells”. (Morris, 2014b) The chip chambers of cell cultures were observed with a phase microscopy (Nikon Eclipse E200) under 40X magnification followed by procedures indicated in part C in the “Primary Culture of Chick Embryonic Peripheral Neurons 2: Observation of Live Unlabeled Cells”. (Morris, 2014b) Five different positions of cell clusters on the coverslips from both control and experimental groups were randomly selected for both control group and experimental group. Single still images of neurons were captured by the same microscopy with Sony DFX-X700 camera via the software BTV v6.0b1 on system Mac OSX 10.5.8.

In this experiment, all long dendrites that extended from the cell body were defined as axons, and the independent triangular cells that did not grow from the nervous cells were defined as glial cells. Only the axons that overlapped with glial cells were observed and measured in this study. If a path change of an axon growth occurred when it overlapped with a glial cell, then the interaction between axon and glial was observed. If there was no change in the axon-growing pathway when it overlapped with a glial cell, then the interaction between axon and glial was not observed. The numbers of axons overlapping with glial cell were counted in both control group and experimental group at each position. The numbers of interaction between axon and glial cell were also counted in both groups. The percentage of glia-axon interaction in each group was calculated by using the numbers of interactions observed in each group divided by the numbers of axons overlapping with glia cells within the according group. A comparison between

the percentages of glial-axon interaction in each group would be preformed.

## Results:

The focus of this experiment was the effect of methyl mercury (MeHg) on axon-glia interactions. Although the neurons grew very slowly in vitro, after changing the recipe of growth medium, the length of axon growth was sufficient enough to conduct measurement and produce analyzed data for further interpretation.

The results indicate that compared to the MeHg pre-treated neurons, the neurons in the control group with normal growth medium have a higher percentage of interactions. Although two different cultures with different cells have different numbers of axons growth, the percentage of axon-glia interaction is comparable. The numbers of changes in axons direction when interacting with glial cells could be observed under the 40X microscopy as indicated in figure 1 and figure 2.

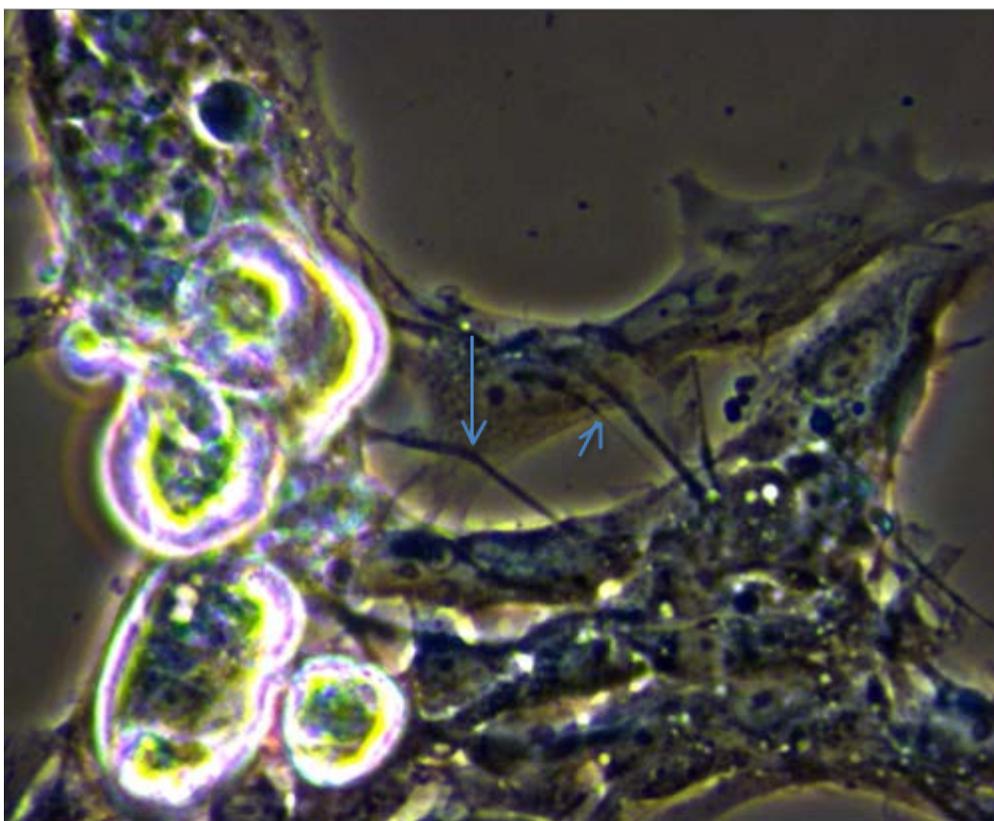


Figure1: Axons interacting with glial cell in the control group. The long arrow in the figure indicates that an axon interacts with the glial cell and causes a direction change. The short arrow indicates that an axon “interacts” with glial cell but does not cause a direction change, which was interpreted to mean the axon and glia actually do not interact with each other.

All the axons that were observed in the cell culture grow in straight direction if the axons were not touching any other cells, such as the axons grew out from other cell bodies, the glial cells, or the other cell bodies around the axon. The axon growth path would change once it “touched” these cells. As a result, the interaction between the glial cell and axon would be observed by detecting the change of axon direction.

Totally, 31 axon-glia overlapping were observed from the single still images taken in five different locations of

the coverslips in control group. 26 of them showed a direction change after the axon touched the glial cell.

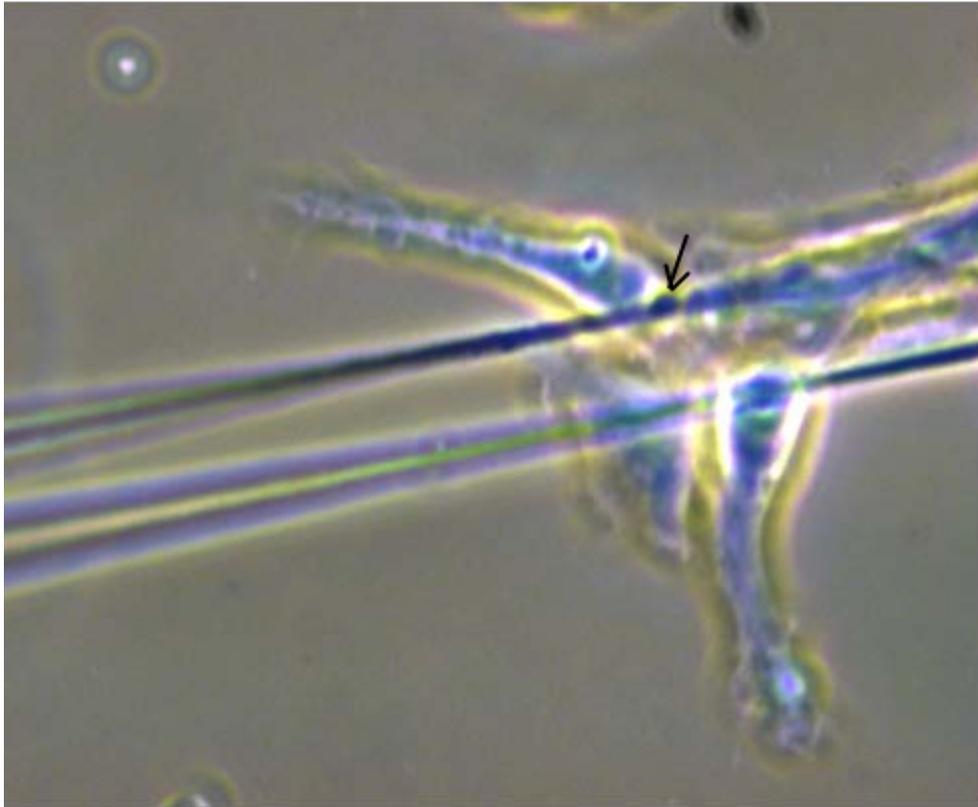


Figure2: Axon and glial interacting with pre-treated MeHg under 40X phase microscopy. The arrow indicates the point where axon and glia “interact” with each other. This “interaction” did not cause a direction change for axon, so that the axon and glial cell were overlapping, but not interacting.

Compare the percentage of axon direction change in the control group, there were much less in the MeHg pre-treated group (figure 3).

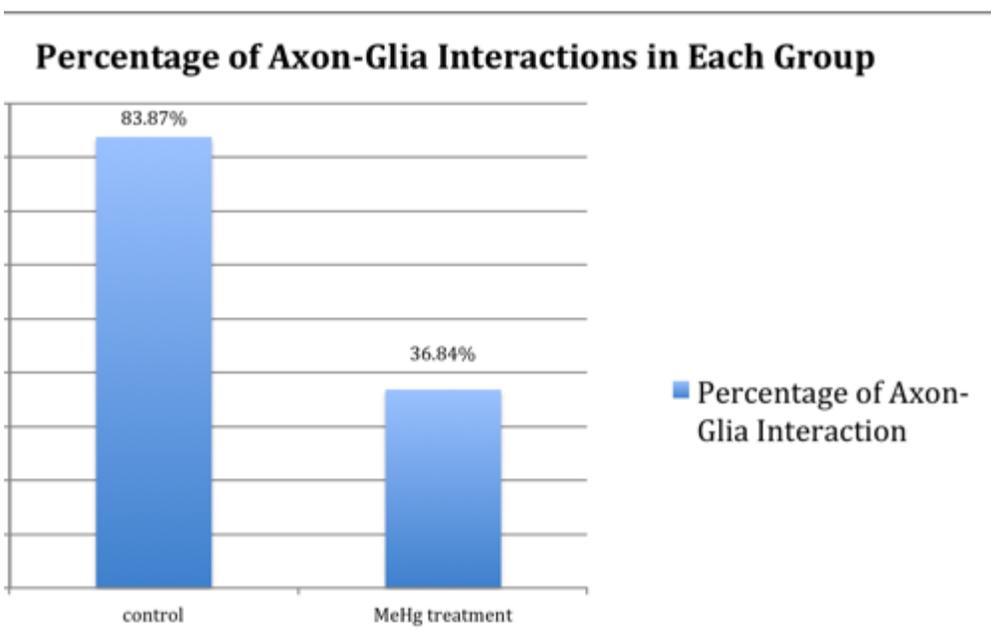


Figure3: Percentage of axon-glia interaction found in control group and found in MeHg treatment group comparison. It

showed a noteworthy advantage of finding axon-glia interactions in control group.

## Conclusion:

In this experiment, the effect of MeHg on axon-glia interaction was studied. According to the previous research, the MeHg would have potent effect on nerve cells and glial cells growth (Stem Cell Therapies, n.d.). Lopachin and Aschner (1992) indicated that the glial-neuronal interaction could influence on the development and regeneration of central neuron system (CNS). Schwann cell could secrete several collagens and proteoglycan, which are the major components in lamina, via the axonal influent. The neurite outgrowth during development and regeneration also depends on glial cells for guidance and stabilization. Based on those studies, this experiment was carried out. After a series of data analysis, the interaction rate between axon and glia in the control group was almost as double as it is in the experimental group. Therefore, my hypothesis, axon-glia interactions would be less under the effect of methyl mercury than that in the normal growing environment, was supported.

Interestingly, there were fewer clusters of neurons growth in the normal treatment group than in the MeHg treatment group. There were fewer interactions observed in the control group than we had expected. In the paper from Lopachin and Aschner (1992), they also mentioned that glial cell exposing to the neurotoxic could cause a cell injury or inhibition of cell growth. MeHg, as one of the neurotoxic were found preferentially accumulate within the astrocytes. From these previous studies, decreased axon-glia interactions under MeHg exposure were expected. Exposure in the neurotoxic environment would prohibit the growth glial cell, so it would be reasonable that less interaction between glial cells and axons were found. If enough same results could be obtained in future experiments, the conclusion would be more convincing.

Also, since in this experiment, glial cells and neurons often formed into a big cluster of cells and cause some difficulties while observing individual axon-glia interactions, it is suggested to decrease the cell culture's density in future studies.

Since very few previous studies have investigated on topic similar to this study, the method of data collection and analysis could be adjusted and improved for further experiments in the future. An experiment done by Ioannidou and his colleagues (2012) previously, which could be a potential reference in the future experiments for using time-lapse imaging instead of single still imaging. In this study, the authors used time-lapse imaging as a fundamental method and studied on the axon-glia interaction *in vitro* and *ex vivo*. Clear changes of axon growth interacting with glial cells were observed over time. In this experiment, we did not include a time-lapse observation for both control group and the experimental group, so that the process of axon growing and axon interacting with glial cells was excluded. Further investigation should be done to address this issue in order to obtain more information about MeHg's effect on axon-glia interaction. Also in our experiment, due to the small size of our data pool, it is inappropriate to make a definite conclusion just yet. Therefore, future experiments with larger data pools are highly recommended.

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