Interactive Occurrences between Glial Cells and Neurons in *in vitro* exposure to Methylmercury

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INTRODUCTION

Methylmercury (MeHg) is an extremely hazardous chemical due to its neurotoxicity. Methylmercury mainly affects the motor and sensory systems, especially the area of sensory motor integration. Like all chemicals, the exposure and susceptibility of the host determine the effects Mahaffey, K. R., 1999).

Populations who predominantly rely on marine life for their diet are at greater risk to methylmercury exposure. Previous research on the effects of methylmercury has been done in Japan. In the 1950's, Japan suffered from a methlymercury epidemic due to their long-term consumption of fish. The industrial waste that was being dumped into the waters of Japan poisoned the fish. A similar incident happened in Iraq in the 1970's when hundreds of people died from methylmercury poisoning because they had consumed bread that was treated with organomercury fungicide (Castoldi, Coccini, Ceccatelli & Manzo, 2001).

Methylmercury poisoning in adults can lead to damage in the visual cortex, motor cortex, and depletion of the granule layer of cells. Prenatal or immature central nervous systems are much more susceptible than an adults' central nervous system. High exposure levels to a fetal central nervous system can result in cerebral palsy, blindness, deafness, and mental retardation (Castoldi, Coccini, Ceccatelli & Manzo, 2001). Even if the mother shows no signs of poisoning the fetus, it is still in danger and vulnerable to this hazardous waste product.

Methylmercury is a dangerous chemical because it can readily cross the blood-brain barrier and cause harm to humans (Allen, Shanker, Tan & Aschner, 2001). Previous methylmercury-high dose in vivo studies have shown that once methylmercury is in the central nervous system; the cell will complete apoptosis (Allen, Shanker, Tan & Aschner, 2001). However *in vitro* studies have shown that methylmercury is mostly found in the glial cells and not in the neurons.

Astrocytes, or glial cells, were named by scientist Virchow for their star-like shape and mainly serve as structural support for the neurons. Virchow also called astrocytes "neuroglia" which translates to nerve glue in German. For over a century Virchow's notion that astrocytes only served as structural support held true. Astrocyte and neuron interactions

are is essential to the cells. They can be exemplified in different ways, for example, when astrocytes release neurotropic factors, when astrocytes are guided through neural migration, as well as astrocytic glutamate uptake and its metabolism to glutamine. Another example of interaction between astrocytes is supplying precursor molecules for neural glutathione synthesis. (Allen, Shanker, Tan & Aschner, 2001).

This study tested the effects that MeHg would have on the glial-neuronal interactions. *Gallus gallus,* or domestic chicken, embryos were used as the subject of this experiment. This study hypothesized that if the primary tissue culture of a *Gallus gallus* DRG's are removed dissected and pretreated in methylmercury, then the number of interactions between the astrocytes and neurons will increase (Allen, Shanker, Tan & Aschner, 2001). I hypothesized that the number of interactions will increase because the astrocytes will act as a protective layer to the neuron from the depleting effect of methylmercury (Sanfeliu, Sebastian & Kim, 2001). I believe this to be true because glial cells are more durable than the neurons; meaning that it takes lower dose and a less amount of time for the methylmercury to effect the neurons opposed to the time and dosage needed to effect the glial calls. Therefore, the neurons are depleted first allowing the methylmercury to get to the neuron. This study is relevant to the public because it examines the danger of methylmercury exposure. Glial-neuron interactions are essential because glial cells support the neuron in many ways. Glial cells provide support and protection to the neuron. They do this by surrounding them and provide structural support, supply nutrients and oxygen, insulation from neighboring neurons, and they remove the dead neurons. Without glial cells out central nervous system would not be able to function, hence, methylmercury is a neurotoxin.

MATERIALS & METHODS

The dissection of 10-day-old *Gallus gallus* chick embryonic peripheral neurons was executed using the materials and procedure for "Preparation Day Steps" protocol from Robert L. Morris (Morris, 2014a), with the exception of the "Flame-constriction of pasteur pipettes" this part of the procedure was not necessary. The "Dissection Day" steps needed alterations. *Coverslip treatment 1* procedure described in Morris, R. L., 2014a, can be followed as instructed with the exception of the time allotted for the cover slip to sit in the poly-K, instead of 20-30 minutes this should be increased to 3 hours. *Dissection Part 1* was done as described in Morris, R. L., 2014a. *Dissection Part 2* was modified in steps 4, 6, and 8. In step 4, 60 ganglia and 6 sympathetic chains were removed. In step 6 the coverslips laid in the poly-K for 3hours, as mentioned above. *Coverslip treatment 2* procedure remained the same. *Coverslip treatment 3* was changed in step 3. The poly-K coated coverslips laid in the droplets of laminin for 2 hours. The growth medium concentration

changed in *Coverslip treatment part 4*, step 5; 200ng/mL of Neuron Growth Factor was used. Follow *Dissociation of ganglia part 1- cells into trypsin* was followed as outlined in Morris, R.L. 2014a.

Plating out Control. Follow as per Morris, R.L. 2014a, with the exception of adding 8 drops of dissociated cells in trypsin into the middle of the coverslip that is already in growth medium. Incubate at 37^{o} for 20 minutes.

Plating out Experimental. Follow as per Morris, R.L. 2014a, with the exception of adding 8 drops of dissociated cells in trypsin into the middle of the coverslip that is already in 40nM MeHg DMEM. Incubate at 37^{o} for 20 minutes.

Density. Follow as per Morris, R.L. 2014a.

Timing. Follow as per Morris, R.L. 2014a, with the exception leaving cells to grow for 38 hours.

Media. Follow as per Morris, R.L. 2014a, with the exceptions of the NGF and glutamine concentrations. NFG 200ng/mL. 4mM L-glutamine.

Age. Follow as per Morris, R.L. 2014a.

Creating a Chip Chamber. Follow as per Morris, R.L. 2014b

Observation and Data Collection:

After 38 hours of growth, the cultured cells were observed in the ICUC imaging lab of the Mars Science Center at Wheaton College, MA. The observatory chip chambers were created by following the procedure located in “Observation of Live Unlabeled Cells” lab by Morris, R.L. (Morris, 2014b). Observations of the slides were viewed under phasing optics on the Nikon Eclipse E200 microscope. The microscope also used a SONY DFW-X700 camera with a C-mount of 1.0x. The field of view connected to the Mac OSX version 10.5.8. monitor using BTV Pro software. The control slide was placed under a microscope at magnification 40x on the Phase 2 setting. A space heater was positioned about 3ft away from the microscope to incubate/heat the cells. If the heater were closer than 3feet it would melt the VALAP, if it were farther than 3feet it would not conduct enough heat for the cells to stay active. Images were collected capturing glia-neuron interactions. Five photos of different glial-neuron interactions were taken of the control slide. Ten photos were also taken of the pretreated MeHg slide at the same magnification, same microscope, and same computer. Three images of the control slide, and three images of the experimental/ pretreated 40 nM MeHg DMEM were chosen for the final data set. They were chosen based on similar situations on the cover slip. The numbers of interactions in the chosen pictures were counted and used to calculate the average number of interactions in a control setting versus the pretreated 40 nM MeHg DMEM setting. These averaged are compared in RESULTS to see if MeHg effects the glia-neuron

interactions.

This study used microscopy to obtain data. Images were analyzed using the following criteria. Glial cells are abundant within the sample of *Gallus gallus* cell culture. Glial cells and neurons were viewed on a microscope at 40x magnification on a phase 2 setting. The neurons had a “phase halo” effect; this is because neurons are not flat but spherical. The cytoplasm acts as a lens and was illuminated when the light was shone through it. Neurons have had a large dark purple spherical cell body, and a long axon when viewed at 40x magnification at phase 2 setting. Nuclei were spotted within the soma of the neurons. Glial cells were defined as the “fried egg” shape. Glial cells grow outward from where they are anchored, they also have a visible nuclei and nucleoli. In Figure 1 you can see the fried egg comparison.

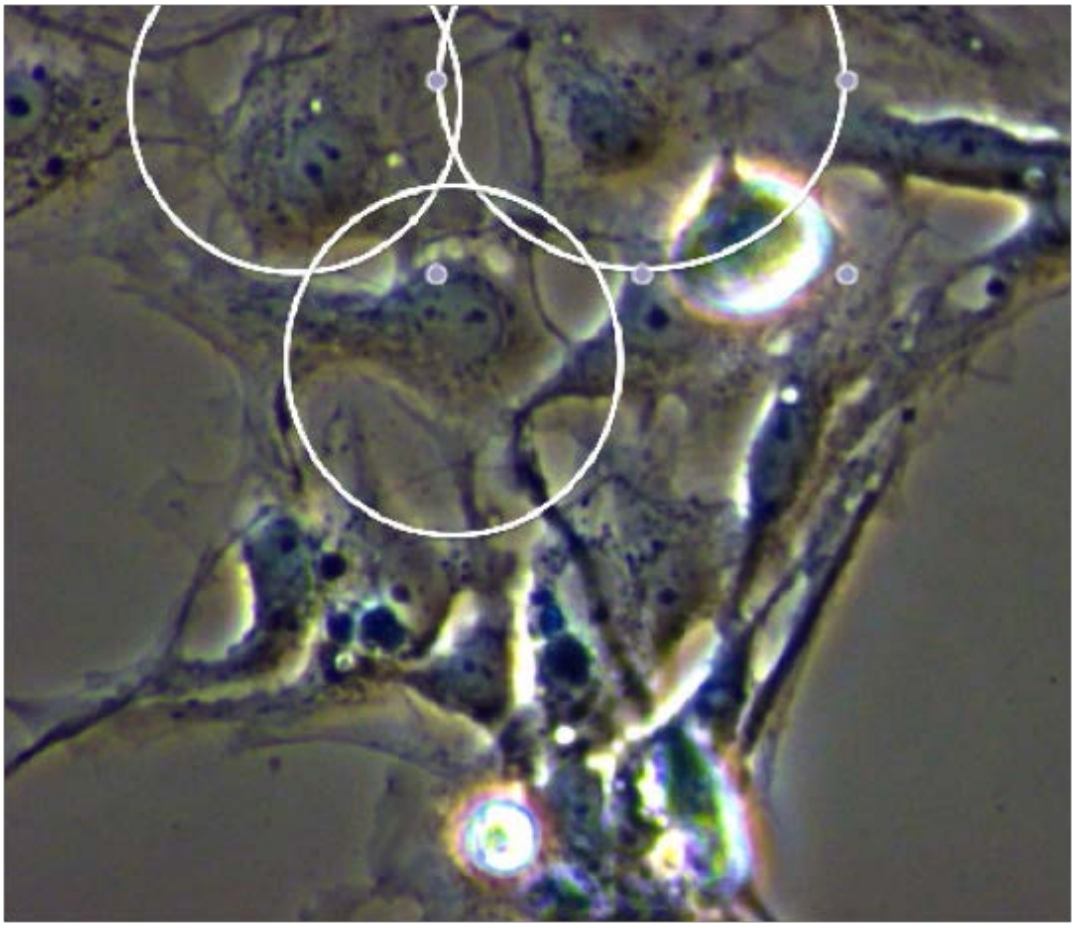

Figure 1. The “egg white” part of the glial cell that was spread thin and long can be seen by the light purple/dark gray outline of the glial cell shape. The circular shape in which the nuclei and nucleoli are located represents the “yolk” of the egg. For this study only, an interaction was described as seen in Figure 2. This picture was gathered in collaboration with Bohan Yang,[1] and Molly Grennell[2].

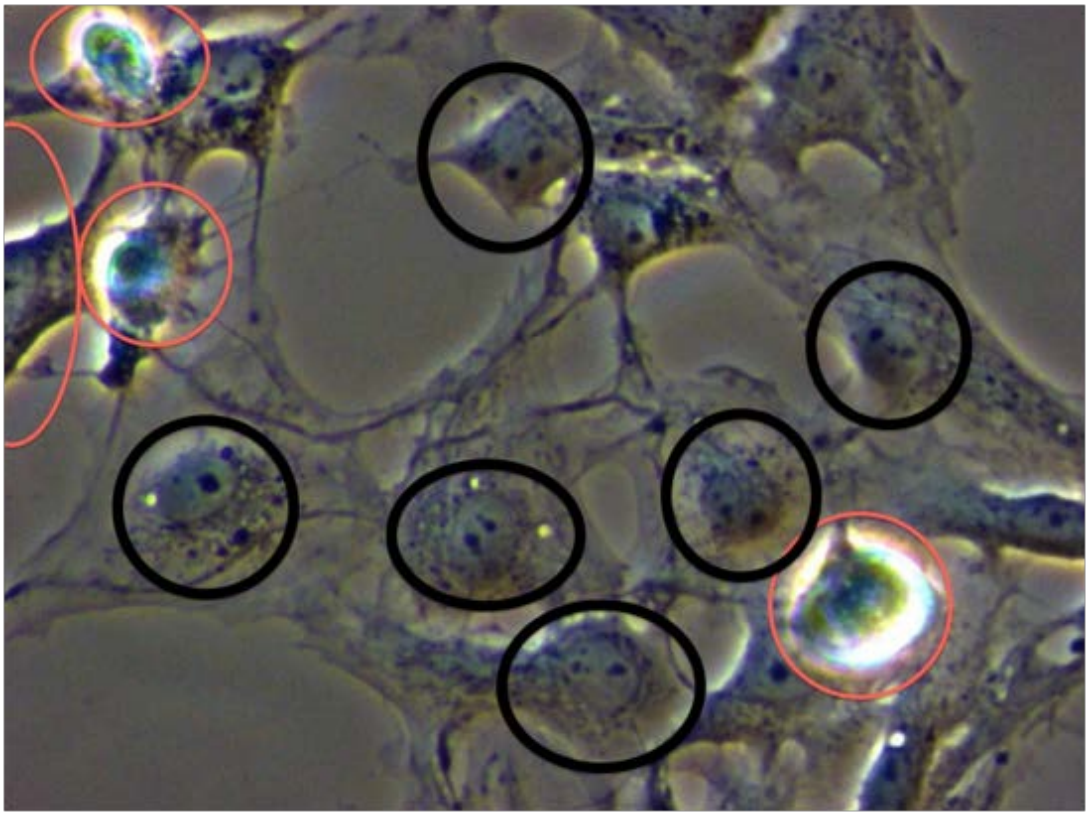

Figure 2. In this picture you can see both black and red circles. The red circles are glial-neuron interactions; the black circles are glial cells that are not interacting with a neuron. Above one can depict that glial cells are spread across the coverslip and that neurons have landed on top of it. The glial cell barriers are roughly boxed off in order to see the glial cell surface area. The neuron cell body is circled in red. This is an example of an interaction. In this experiment a neuron- glial interaction is defined as the attachment of a neuron to a glial cell in such a manner than when placed one on top of the other, no portion of the neuron resides outside the dimensions of the glial cell. If the cell body of a neuron is anchored on the edge, or half way off of a glial cell that will not count as an interaction. To be considered an interaction the neuron must fully sit on the glial cell. The visible barriers of the glial cell can determine this. This picture was gathered in collaboration with Bohan Yang, [1] and Molly Grennell[2].

RESULTS

The pictures used for the data set show that glia and neurons interact more when pretreated with methylmercury, MeHg. The pictures can be seen in Figure 3, 4, 5, and 6.

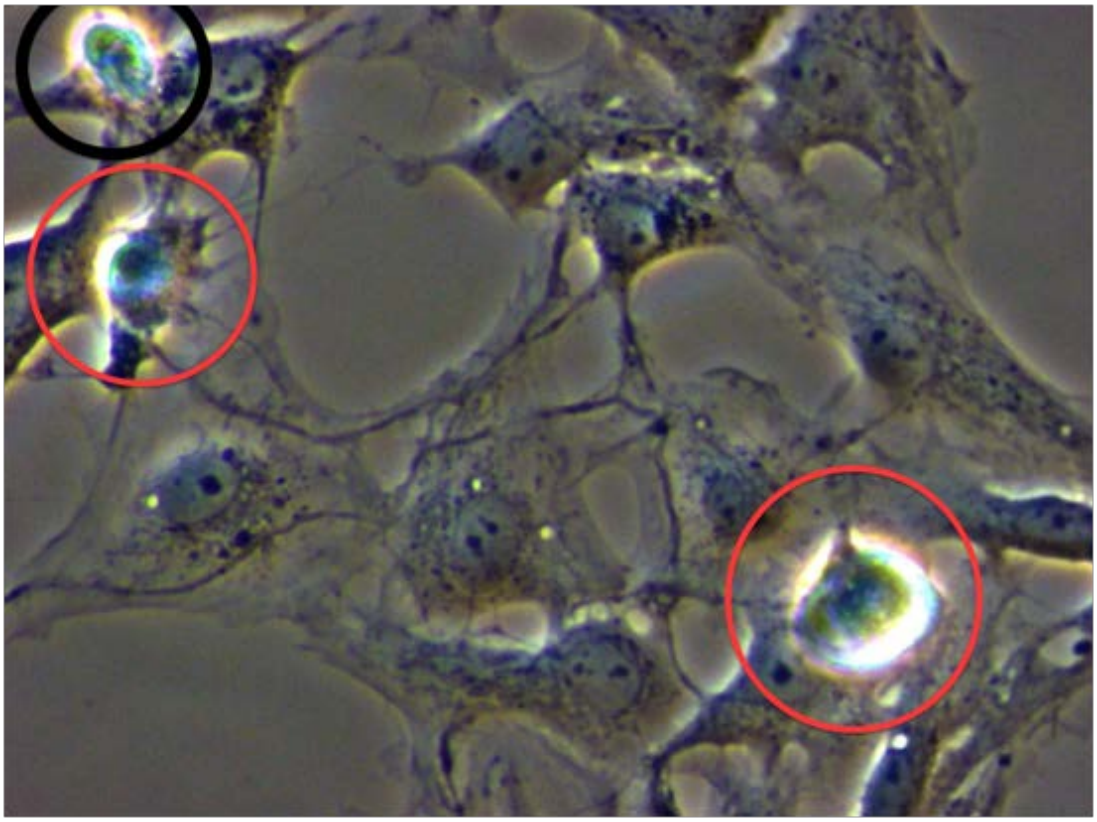

Figure 3. Control Picture 1. This is a picture of a cluster of cells in a control sample. There are two neuron-glial interactions that are circled in red. The neuron that is circled in black is not considered a neuron-glial interaction because some of the neuron is not on top of the glial cell. This picture was gathered in collaboration with Bohan Yang,[1] and Molly Grennell[2].

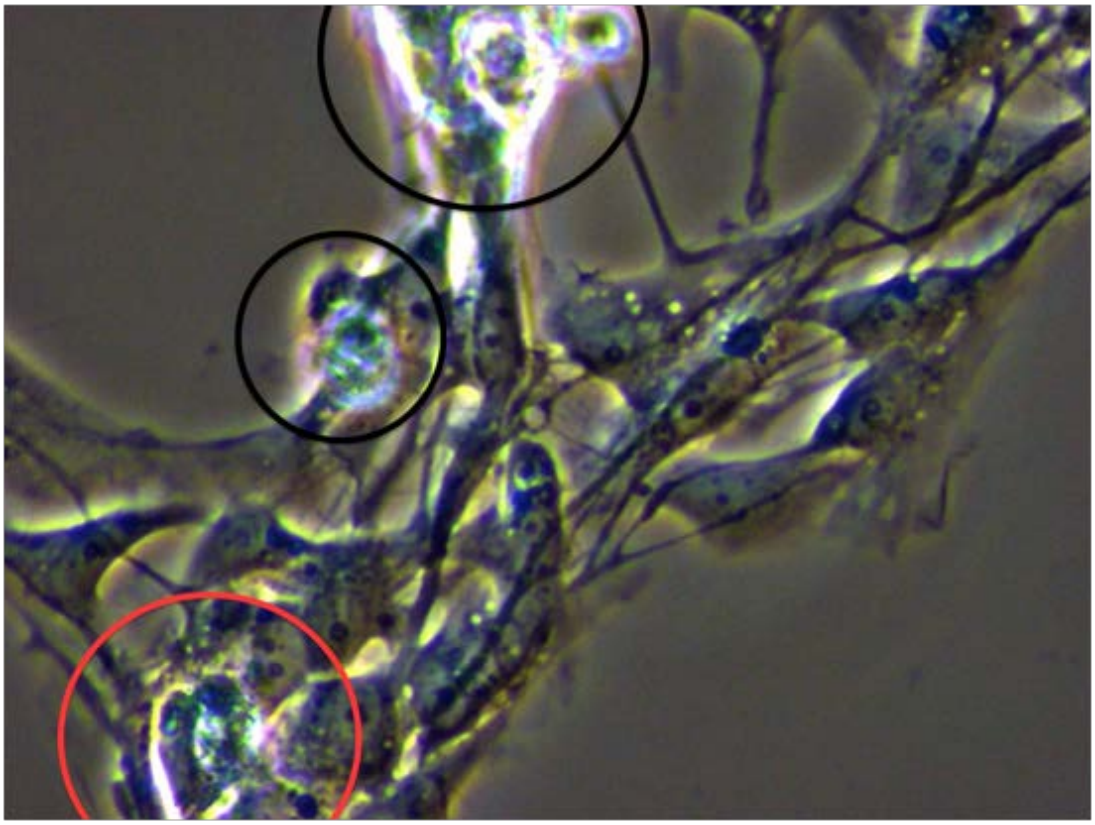

Figure 4. Control Picture 2. This is a control sample. There was one neuron-glial interaction recorded, circled in red, because the other two neurons, circled in black, are not fully on the glial cell.

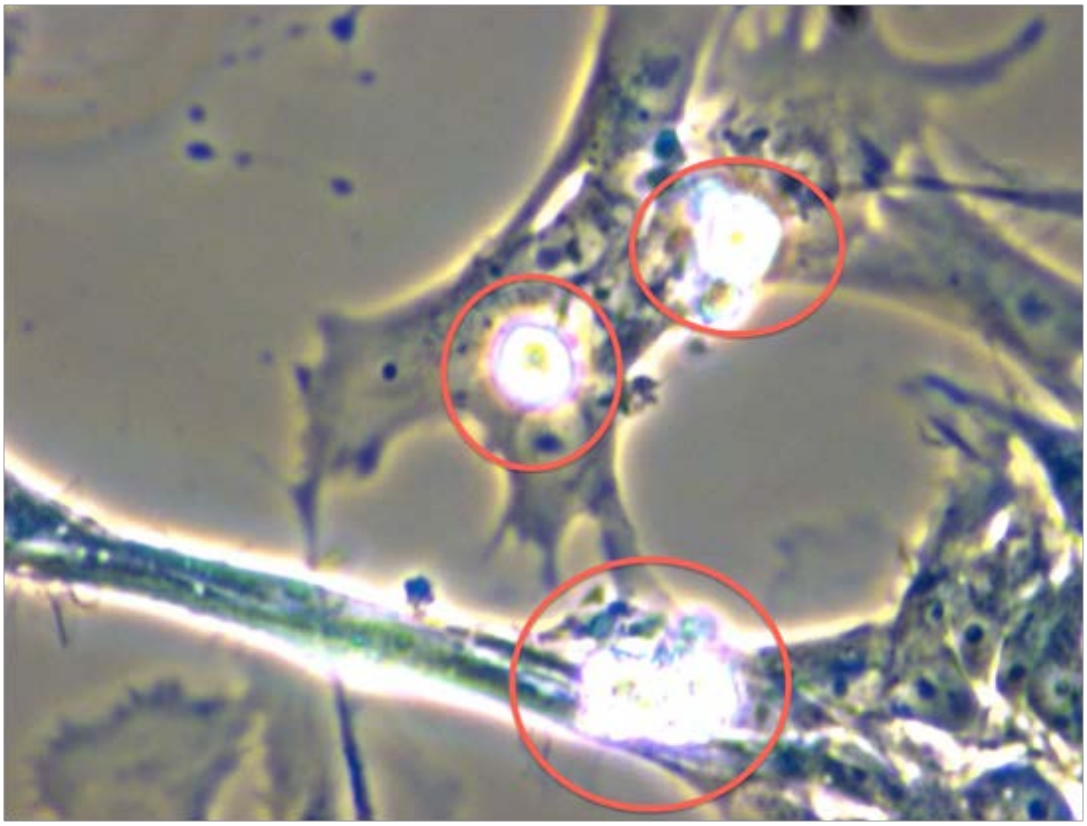

Figure 5. Experimental 1 pretreated with 40nm MeHg DMEM. This cluster of cells was pretreated with MeHg. There are

three glial-neuron interactions indicated here by the red circles.

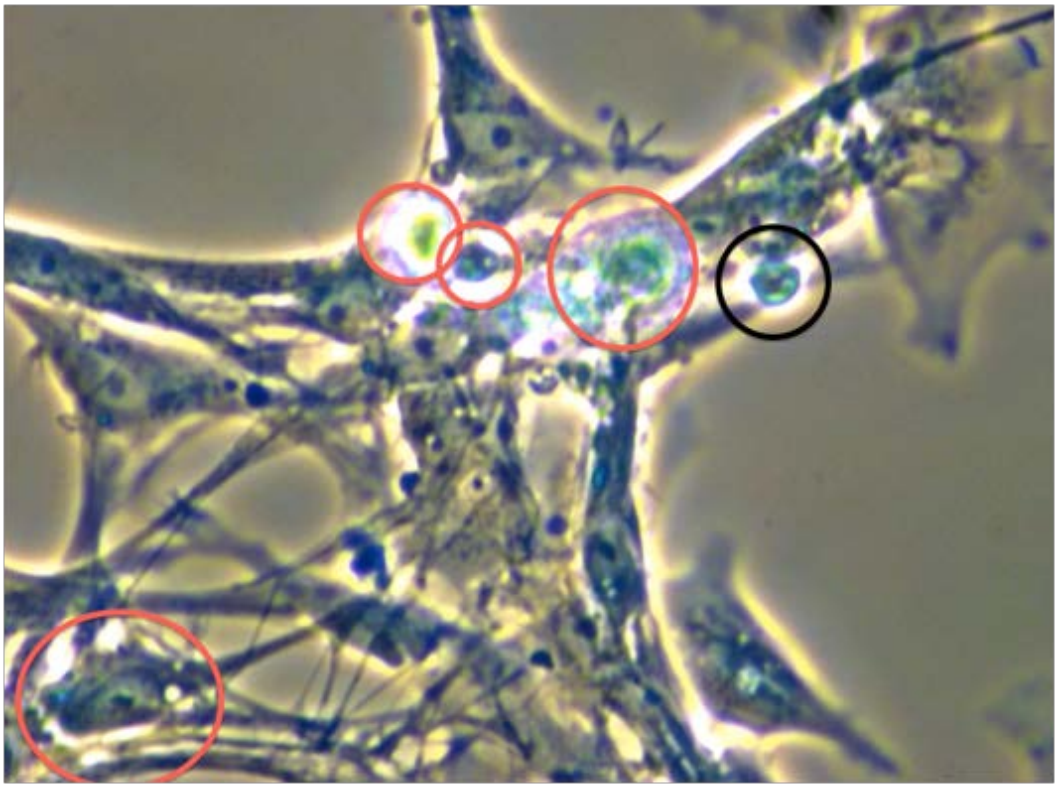

Figure 6. Experimental 2 pretreated with 40nm MeHg DMEM. This cluster of cells had four glial-neuron interactions. Four of the five neurons in this picture were completely on the glial cell, (circled in red). The neuron circled in black hangs off of the glial cell area.

The embryonic chick cultures that were pretreated with MeHg were more active all over the coverslip. Apoptosis was expected with the MeHg cultures but was not seen during this study. The glial-neuron interaction occurred more in the MeHg sample. This can be seen in Figure 7.

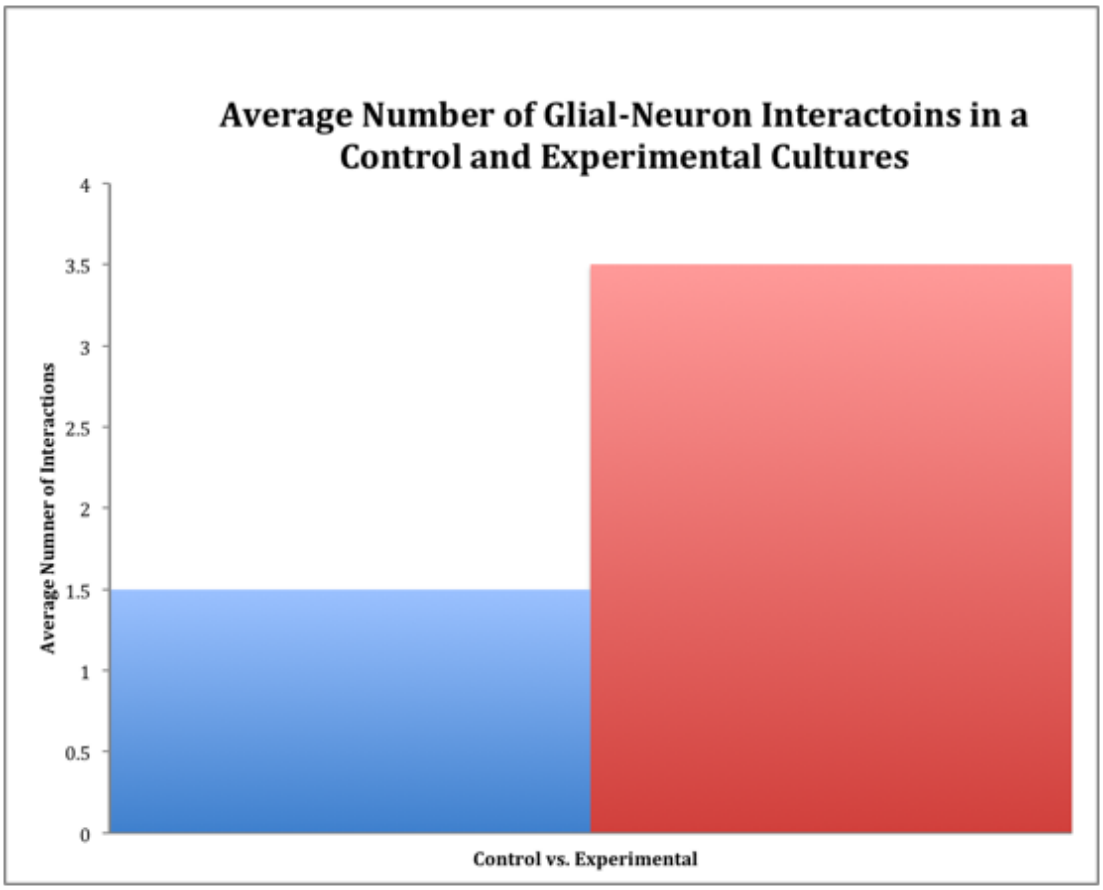

Figure 7. A graphical representation of the average number of glial-neuron interactions calculated from the control and experimental groups. The sum of glial-neuron interactions in the control group was added and divided by 2. The same procedure was used for the experimental group.

DISCUSSION

The data collected supports the hypothesis that number of glial-neuron interactions will increase if treated with methyl mercury, MeHg. I believe that the number of glial-neuron interactions increased because of the methylmercury that was added to the culture. . This allowed for the cells to successfully grow in culture. (Sanfeliu, Sebastia & Kim, 2001). Methylmercury is a neurotoxin; its activity can be characterized by the ability to inhibit neuronal control over the ion concentrations across the cell membrane (Crespo-Lopez, M. E, 2006).

In this experiment two individual samples were used, one for control and one pretreated methyl mercury sample. To do this experiment in the future one should make the following changes. The first change would be only using one sample. To obtain most accurate results only one cluster of glial-neuron should be studied using a flow chamber. Using one sample or one cell cluster would make it possible to observe the immediate and long term effects that

methylmercury has on glial-neuron interactions. Due to the struggle that the class had growing long axons, the observation period would have to last for 48-72 hour duration to allow the axons to grow. The best way to watch this would be either a series of time lapse pictures taken every minute or so, or with a movie. The method I suggest to use would be to observe a control coverslip slide, then execute a buffer change with the 40nM MeHg DMEM and observe for 48-72hours. I believe that a single culture would be beneficial because the experimenter would be able to observe how the cells are moving in a control setting. Then after the buffer change, one would be able to observe the short term, and long-term reaction of the cells. Also one would be able to observe before and after pictures to measure the exact changes.

The experiment that was preformed has flaws, one being the multiple sample data. The control and experimental pictures used in this experiments data set were chosen at random and were compared because they simply looked alike. If I were to use a single sample and focus on one cluster of cells I will be able to follow the mechanism for each change and further understand the effects that methylmercury has on glial-neuron interactions.

There was much room for human error in this lab. The pictures used for the data set were useful but biased. My hypotheses, data analysis, and topic changed after comments were given and I had to make the most out of what I had. This lab is replicable, and the data supports my hypothesis, however I am open to see what other scientists conclude in further trials.

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I have abided by the Wheaton College Honor Code in this work.

Collaboration and images were shared with Bohan Yang[1] and Molly Grennell[2].

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