I. Introduction

Lamellipodia are thin, sheet-like protrusions found at the leading edge of motile cells, such as neurons. These structures are composed of dense actin filament cross-links that extend between fingerlike projections known as filopodia (Rocher, 2000). These veil-like extensions involve the polymerization of actin filaments coupled with translocation of the plasma membrane (Roucher, 2000). Lamellipodia expansion is a natural characteristic, which appears as an outward rippling effect.

Mercury is a highly controversial neurotoxicant that has demonstrated significant destruction of microtubule formation. In a review of 58 studies, 43 attribute mercury to the autism spectrum disorder (ASD), a neurological condition that finds children with ASD more likely to be disposed to heavy-metal intoxication than normal developing children, and that with greater metal intoxication in the child results in greater severity of symptoms (Kern, et al., 2012). Mercury has been shown to depolymerize tubulin, which normally polymerizes to form microtubules and support the structure of axons and dendrites. Guanosine triphosphate (GTP) provides energy that facilitates this process, however, if mercury is present, it will bind to the GTP binding site of the tubulin beta subunit proteins, inhibiting GTP input, and thus, polymerization (Kern, et al., 2012). As microtubules break apart, axons and neurites collapse and degenerate (Kern, et al., 2012). A study in 2010 showed evidence of the retraction of processes or lack of axons in autism, describing it as “impaired neuron activity” Kern, et al., 2012).

It is important to understand the effects of mercury on the vast properties and behaviors of nerve cells. Lamellipodia in the presence of neurotoxins are not well researched, which has motived this research project. The experimental organism the domestic chicken Gallus gallus, in embryo stages serves as an excellent lab organisms for neuron dissection. It is very difficult to humanely retrieve living neuron cells. G. gallus incubated for 10 days is long enough for the embryo to form nervous system cells, but too early to develop a response to pain (2/13/13 (1) LJH). Therefore, the neuron cells are cultured under healthy and morally sound conditions.

This study tested the hypothesis that in the presence of mercury, the abundance of lamellipodia in embryonic
chick neurons would be fewer than in a non-mercury environment. This experiment treated a flow chamber of cultured nerve cells in a controlled environment using Tyrodes Salt Solution and in an experimental environment using 100nM of mercury solution. The cells were viewed using time-lapse phase microscopy.

II. Materials and Methods

Dissection

Procedure adopted from protocol customized by Professor Robert Morris. See “Primary Culture of Chick Embryonic Peripheral Neurons 1: DISSECTION” protocol (2013). The cells were 35 days old. Hanks Balanced Salt Solution (HBSS) was used instead of Dulbecco’s Modified Eagle Medium (DMEM).

Flow Chamber


Imaging and Introduction of Mercury

The following imaging steps were conducted in the Imaging Center for Undergraduate Collaboration at Wheaton College, Norton, Massachusetts. A Mac OS X computer (Taurus) was used in conjunction with a Sony digital interface (DFW-X700) camera on the Nikon EFD-3 light microscope. The materials used for this experiment imaging included a heater, thermometer, 1 ml of Tyrodes and 1ml of 100nM of mercury. At roughly 37°, the flow chamber was observed and aligned for Koehler illumination at 40x magnification. Four drops of Tyrodes was flowed through the flow chamber. Once the chamber was well saturated, imaged were captured every 15 seconds for 15 minutes. This procedure served as the control. The experimental procedure introduced several drops of mercury to the flow chamber and a series of images were captured every 15 seconds for 15 minutes. Data was compiled into time-lapsed microscopy movie for further analysis. The same neuron was viewed for both pre- and post-mercury exposure. Photographs were observed in the program BTV.

Data Collection

The data used in this study was obtained by collaborators, Michaela Superson, Corey Laliberte, Magan Laliberte, and Cailin McCloskey. By viewing the time-lapse microscopy of the pre-mercury exposed neuron at each minute marker over the span of fifteen minutes, the numbers of lamelliopodia were quantified. Using Image J, any
lamellipodium with an area of 396 pixels or greater were accounted for, therefore, it is important to note that not all
lamellipodia were accounted for. These numbers were represented in a line graph. The average lamellipodia counts
were also calculated for both pre- and post- mercury treatments. In addition, motility was measured by cellular material
that had traveled 442 pixels or greater in distance.

III. Results

Observations

In pre-mercury exposure, the cell body moved about 602 pixels over the span of fifteen minutes. This motility behavior can be observed by comparing Figure 1 and 2. The post-mercury exposed neuron presented fewer lamellipodia, as seen in Figure 3 or 4. Additionally, there was much less expansion and downward axonal movement. No neuronal motility was observed. Overall, more axon retraction and extension was observed in the pre-mercury exposed neuron. Lamellipodia was surprisingly facing away from the direction of movement (based on the previous observations directionality).

Data Analysis

The average number of lamellipodia found in the pre-mercury exposed neuron was 3.3, while for post-mercury exposure 1.7 (n = 1). As seen in Figure 4, there is a dramatic difference in lamellipodia abundance between the pre- and post-mercury treated neuron. The stark fluctuations in numbers were somewhat consistent between both treatments, as Figure 4 displays an almost synchronous increase, decrease effect in lamellipodia. Other than at minute 3, there is a consistent increase in lamellipodia in the pre-mercury exposed neuron.
Figure 1. Minute 1 of pre-mercury exposure. This image depicts the pre-mercury exposed neuron with four distinct axons. Because the third from the top left retractcd immediately, it was not counted. There was some lemmellipodia activity visible on the third axon. This area remains active with lamellipodia over the span of fifteen minutes.
Figure 2. Minute 15 of pre-mercury exposure. This image depicts the pre-mercury exposed neuron after fifteen minutes. The cell body compacts itself closely to the shortened axons. There is an abundance of lamellipodia near axon three.
Figure 3. Minute 15 of post-mercury exposure. This image depicts the post-mercury treated neuron at the end of the fifteen-minute interval. Only two lamellipodia were counted in this diagram. Axon length appeared shorter and less dense in comparison to pre-mercury exposure.

Figure 4. Comparison of lamellipodia abundancy pre- and post-mercury exposure. This graph depicts the number of counted lamellipodia in pre- and post-mercury treatments over a period of fifteen minutes. It is evident that the pre-mercury exposed neuron had more lamellipodia (under the defining conditions) than the post-mercury treated neuron.

IV. Discussion and Conclusion
This study demonstrated the increase in abundance of lamellipodia in the pre-mercury exposed neuron than the post-mercury exposed neuron, supporting the original hypothesis. In the pre-mercury exposed neuron, axon three showed prolific polymerization of lamellipodia, forming one large clump. It is well understood that the forces generated by actin filament cross-links at the leading edge induce membrane protrusion and subsequent lamellipodial growth (Molinger, 2012). The fluctuations seen in Figure 4 can be explained by the natural occurring polymerizing and depolymerizing behavior of a lamellipodium. Overall it was apparent that lamellipodia in the post-mercury exposed neuron did not span the axons, as was observed in the pre-mercury exposed neuron. There was no blebbing or sign of apoptosis, however, the low numbers and static behavior lamellipodia was evident. In conclusion there was a decrease in abundance of lamellipodia in the post-mercury exposed neuron.

Membrane tension is caused by in-plane tension in the lipid bilayer and adhesion between the membrane and cytoskeleton (Keren, 2011). The membrane tension exerts a force around the cell that affects processes like motility (Keren, 2011). According to a study, conducted by Ellen Batchelder, on membrane tension and regulation of motility, there is a relationship between lamellipodia abundance and membrane tension. Cell movements occur by a crawling mechanism in which polymerization of the cytoskeletal protein actin pushes out the leading edge membrane. Relaxed membrane tension in Caenorhabditis elegans sperm cell via osmotic shock and deoxycholate treatments depolymerized the membrane, resulting in a lack of adhesion under the lamellipodium. These findings suggested that membrane tension reduction may cause a decrease in speed of cell movement, therefore an increase in membrane tension increases motility (Batchelder, 2010). Polymerization rates may follow a similar trend. Membrane tension reduction results in unorganized, rough lamellipodia, composed of short filaments angled away from the direction of movement (Batchelder, 2010), as was also seen in the results of the post-mercury treated neuron.

This study suggested that increase in tension reduced lateral membrane protrusions in the lamellipodium, and filaments were longer and more oriented in the direction of movement (Batchelder, 2010). Membrane tension optimizes motility by forcing polymerization in the direction of movement, thus functioning as a directionality regulator (Batchelder, 2010). It can be speculated that a similar depolymerizing mechanism is seen between mercury treated neurons and osmotic shock and deoxycholate treated cells. Both studies presented results in decreased motility due to the disassembly of actin, thus creating a lack in membrane tension. In conclusion, polymerization rates may follow a similar trend in the presence of mercury.

There is perhaps another interesting connection between the lamellipodium of the chick embryo and sperm cells. Because the sperm cells contained only cytoskeleton, mitochondria, and nuclear material, Batchelder inquired whether or not this phenomenon would be applicable to actin-containing cell types as well (Batchelder, 2010). The chick
embryo data suggested it in fact could apply to these cell types, though further experimentation and duplication of this experiment would be required.

In addition to more research on this correlation to membrane tension, a more objective parameter could be applied in determining which lamellipodia were counted and which were not. Lamellipodia that measured 396 pixels in area or greater fit the criteria as a well established lamellipodia, but reducing this parameter may increase the count (data), offering wider range of results. Though the lamellipodia accounted for were roughly measured within each image, this procedure was not entirely devoid of subjectivity. Future studies would observe these same effects in more than one neuron in order to increase the sample size.

V. References Cited


Morris, Robert L. “Primary Culture of Chick Embryonic Peripheral Neurons 1: DISSECTION.” Wheaton College, Norton, MA.

Morris, Robert L. “Primary Culture of Chick Embryonic Peripheral Neurons 2: OBSERVATION OF LIVE UNLABELED CELLS.” Wheaton College, Norton, MA.