The Effects of Mercury on Primary Culture Chick Sympathetic Nuerons: Growth Cone Activity

Introduction:

Neurons are cells found in the brain and body with a specialized function of receiving and transmitting information (Kandel et al. 2000). Neurons have outgrowths known as axons and dendrites. The axon can be thought of as the arm of a nerve cell and each cell has only one axon. The length of the axon varies depending upon its location; for instance, an axon in the brain would be relatively short while an axon in another part of the body could be up to three feet long. Axons transmit outgoing signals from one cell body to another (Kandel et al. 2000). Dendrites are extensions form the neuronal cell body that carry information into the cell body. A singe nerve cell may have many dendrites.

The growing tip of an axon is known as the growth cone. It is the site where new material is added to the axon (Kandel et al. 2000). The growth cone acts as both a sensory structure, receiving directional cues from the environment, and a motor structure, elongating the axons based on its activity (Kandel et al. 2000). The growth cone is broken up into three main regions, the central core, filopodia and lamellipodia (Kandel et al. 2000). The central core is composed of microtubules, mitochondria, and other organelles (Kandel et al. 2000). The filopodia are long, thin extensions from the body (Kandel et al. 2000). Lamellopodia are located between the filopodia and contribute to the motility of the growth cone (Kandel et al. 2000).

In large part the sensory capability of the growth cone depends upon filopodia, actin-rich, membrane-limited structures that are highly motile (Kandel et al. 2000). Within the membrane receptors for molecules that serve as directional cues for the axon are located (Kandel et al. 2000). It is the job of the filopodia to sample the environment ahead of the central core (Kandel et al. 2000). The flexibility, size, and rapidity make filopodia ideal for making detailed inventory of the environment (Kandel et al. 2000). The growth cone is stimulated to advance, retract, or turn when receptors on filopodia encounter signals in the environment (Kandel et al. 2000).

Mercury is a naturally occurring metal which has several forms, liquid and gas (ATSDR 1999). It can also combine with other elements, such as chlorine, sulfur, or oxygen, to form inorganic salts (ATSDR 1999). This inorganic salt form is the one used in this lab. Typically, inorganic mercury enters the air from mining or ore deposits, burning coal and waste, and from manufacturing plants (ATSDR 1999). Another form of mercury most commonly found in the environoment is methylmercury, which is produced by microscopic organisms in the water and soil (ATSDR 1999). Leong et al. (2000) report that methylmercury is a potent neurotoxin that affects microtubule integrity during central nervous system (CNS) neuronal development. Braeckman et al. (1997) also

reports that mercury is a highly toxic environmental contaminant that is a severe hazard to public health as well as other life forms. Because mercury has such an impact on health, it is the aim of this study to determine the effects of filopodia activity after exposure to mercury chloride. I hypothesize that filopodia activity will decrease when exposed to mercury because it is such a strong neurotoxin.

The examination of filopodial formation will indicate how the neuronal cell is responding to mercury. As previously stated, filopodia are responsible for scouting out the area ahead and reporting back to the cell that is either okay to proceed ahead or, the cell should look in another direction (Kandel et al. 2000). Because mercury is such a strong neurotoxin and has been found to cause human disease, it is important to understand how neuronal cells respond to mercury so that eventually a way to protect or cure people from mercury exposure can be determined. In addition, previous research by Leong et al. (2000) has shown that water snail, *Lymnaea stagnalis*, growth cones collapse when exposed to mercury. Therefore we can branch off of their research to determine the effects in chick sympathetic neurons.

Because the project was a classroom experience, I was able to collaborate with several classmates. Amy Silverio and Sara Tower both conducted experiments that were pertinent to mine. Amy reviewed the presence of actin in neuronal cells when exposed to mercury. This is relevant to my experiment, because filopodia are actin-rich structures, therefore, the amount of filopodia present would correlate directly to the amount of actin. Sara was looking at neuron-neuron interactions and quantified it based on the number of branches she saw, which were represented by filopodia. If Sara were to see more branching, then she would be seeing more filopodia. Therefore, all three experiments are interconnected because the more branching, the more filopodia, and the more actin.

External factors affect the development and growth of axons and dendrites (Bray et al. 1987). It is the aim of this study to determine the effect of mercury on the activity of growth cones in primary cultures of chick sympathetic neurons.

Materials and Methods:

The experimental substratum used contained 10nM mercury chloride. Mercury chloride is used because it is a non-polar molecule allowing it to cross membranes. However, it immediately binds to protein so the growth medium cannot be used as a substrate due to the amount of protein in solution. Instead, HBSS was used. After exposure to the mercury chloride solution (20 minutes at 37 degrees Celsius), the neurons were allowed to recover in the F^+ medium for ten minutes at room temperature. However, it is important to note that in order to keep the mercury in solution; it must also be in acid. Therefore 10μM mercury chloride was diluted in 0.5% hydrochloric

acid. The control plate is exposed to a 1:1000 dilution of 0.5% hydrochloric acid in HBSS.

The chick eggs were dissected anywhere from 9-11 days old and were plated in 0.5x, 0.75x, 1x and 3x densities. For this experiment 1x density plates were used unless otherwise noted. I performed the experiment three separate times but quantified the data from one of those trials due to the quality of the images in the other trials.

To collect the data I used the camera, Sony Digital Interface DFW-x700, attached to the microscope at 100x. Use the heaters to keep the neurons alive and growing at 37 degrees Celsius when viewing them under the microscope. Using the Spot! program on some computers it is possible to take a time-lapse video and photos of the growth cone advancement. I took 2 minute time-lapse videos, with 3 frames per second. To quantify my data I looked at the number of filopodia present in an area on the growth cone. I defined filopodia as any thin extension from the main growth cone. I captured frames from videos of a control sample and an experimental. Then, using the Image J program I was able to analyze the number of filopodia on the growth cone in each frame. I determined the number of filopodia by counting any thin extension from the growth cone. I was careful not to include any portion that looked like it was a separate extending growth cone, which was determined based on size, length, thickness, and offshoots. Any section that was deemed to be longer, thicker, and containing other offshoots (filopodia) was not counted as a filopodium. The image below is an example of filopodia, note the offshoots from the main axon.
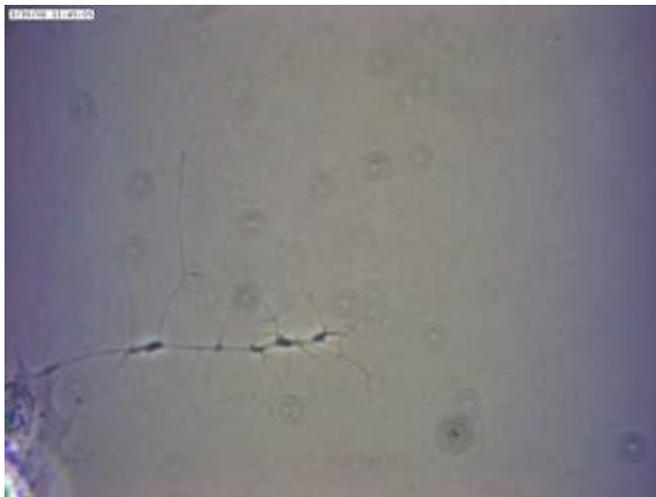

Figure 1: Example of filopodia. Control 1x density.
100x magnification.

In addition, it is important to note that all changes in filopodial shape between frames were counted as a filopodium. I prepared the data in a table so that it could be plotted (number of filopodia vs. time).

Results:

While collecting the data I noticed that the neurons exposed to mercury tended to appear as a clump- they were more tightly woven, whereas the control neurons were more spread out. In addition, the length of the growth cones differed between experimental and control groups. The experimental growth cones were much shorter and tended to look stubby at the ends with many branches of filopodia. On the other hand, the control groups had long growth cones that had obvious signs of lamellipodia and hardly any branchings of filopodia. See the images below.

Figure 2: Control sample 1x density 100x magnification.
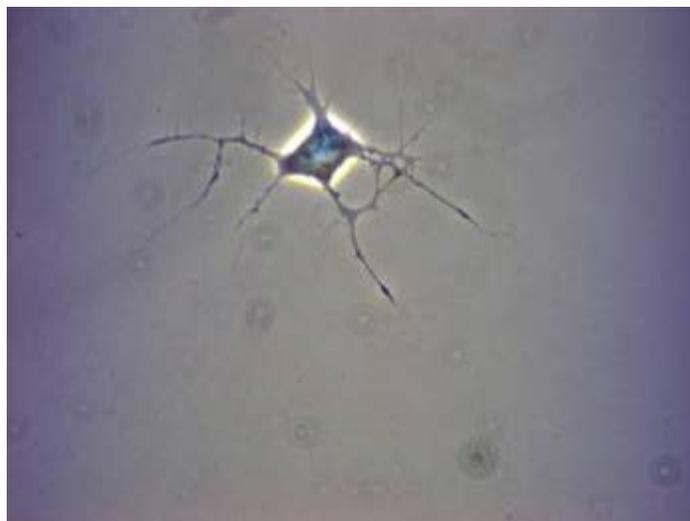Note the lamellipodia and long axons.

Figure 3: Experimental sample 1x density 100x magnification.
Note the short dendrites.

After collecting the images and time lapse movies, I quantified my data using the methods outlined in the previous section. The numbers represent filopodia, so a 6 would mean that there were 6 filopodia present in that frame. I found that in the control conditions there was more filopodial activity, represented by the extension or retraction of a filopodium.   The experimental conditions resulted in less filopodial activity.
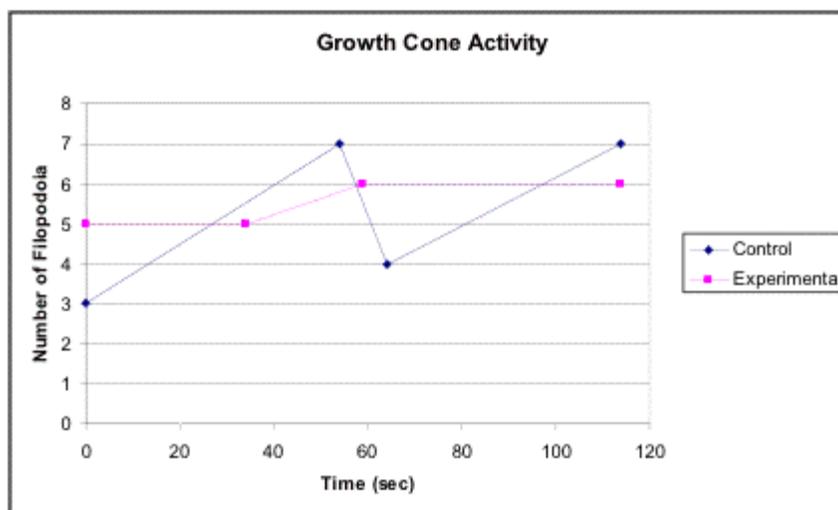
Figure 4: Direct measurement of the formation of filopodia, in both a control sample and a mercury exposed sample. Measurements were taken by recording the number filopodia in a certain region of the growth cone over a period of time. This gives an idea of the degree to which the growth cone is changing shape.

The figure above visually shows the increased formation of filopodia in the control samples. The experimental samples did not exhibit much change, but stayed very constant in their amount of filopodia.

Discussion:

The results gathered indicate that filopodial activity decreases when exposed to mercury. This means that mercury had an affect on the integrity of the neuronal cell which caused it to reduce the amount of branching out in search of places to grow towards. The control samples were actively searching for places to grow towards, whereas the experimental samples were just frozen in place. If a healthy neuronal cell were defined based upon its ability to grow and spread to new areas, then the mercury exposed sample would not be considered healthy because it is not growing or spreading.

Applying the filopodia data to the growth cone as a whole, indicates that growth cone activity decreased when exposed to mercury. Because filopodia are the trailblazers for growth cone advancement, their formation is indicative of how hard the cell is trying to find new ways to branch out and grow. The decreased filopodial formation in mercury exposed samples shows that the growth cone is not as active (growing) as it is under control samples. In the control sample the filopodia are continuously extending and retracting looking for new places to grow towards. My hypothesis that filopodial formation would decrease was supported.

Sources of error in this experiment would include inconsistent heating of the neurons when viewing under the microscope. If the neurons were allowed to reach room temperature they would stop growing and thus, measurements of growth cone activity would be null. This happened during my first attempt at the experiment.

Other sources of error would be counting an extension as a filopodium that is not actually filopodial. That was most likely the greatest source of error in this experiment because it was very difficult and subjective what was labeled a filopodium.

The collaborators, Amy and Sara, also saw similar effects in their data. For instance, Amy saw that actin levels increased (as a function of fluorescence) in mercury exposed neurons (Silverio 2006). This correlates with my data because the filopodia, which are actin-rich, were not growing and extending but remaining constant, so at any given time there would be a greater amount of filopodia and thus actin present. Sara found that when neurons were exposed to mercury there were fewer neuron-neuron interactions and an increased frequency of triple points that resulted from filopodial branching in the mercury exposed sample (Tower 2006). In addition, she also saw an increase in filopodial branching represented by the frequency of triple points. This is indicative of the fact that filopodial numbers remained constant in my data, because the amount of flipodial branching increased. Whereas in her control sample this frequency decreased which relates to the fact that there was greater filopodial activity at any given time.

To refine this experiment for future studies, I would have collected more data from a greater number of neuronal cells. This study is limited because it only looks at the affects of mercury on one sample. In addition, it would be helpful to include data from different aged chick eggs. This data would offer insight into the integrity of the cells at various stages in development. Furthermore, using different concentrations of mercury chloride would shed light on how the neurons respond to various concentrations of mercury.

Works Cited

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