

The effect of mercury on the rate of maturation of macropinosomes becoming lysosomes inside glial cells

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Introduction:

Glial cells are found inside the central nervous system of an organism. They have four main functions and those are: to surround and support the structure of neurons, to supply nutrients and oxygen to neurons, to insulate one neuron from another neuron, and to destroy and remove the damaged neurons from the central nervous system. Michaud (2013) claimed that glial cells that were once used merely for the “housekeeping” of the central nervous system are now found to be essential for the complexity of the communication between cells in the human brain.

Macropinosomes are phase-bright organelles that form very close to the edge of cell’s ruffling edge in growth-factor-stimulated cells (Kerr et al., 2006). They are large in size for particles inside a eukaryotic cell; their diameter is generally less than 0.5 μm (Swanson and Watts, 1995). macropinosomes form in the membrane ruffles because that is where macropinocytosis takes place. “Macropinocytosis is the engulfment of large volumes of extracellular fluid at the base of cell membrane ruffles. It is a process fundamental to the maintenance of cellular homeostasis and is a pathway that is readily exploited by invading pathogens (Kerr et al., 2006).” As macropinosomes mature, they eventually fuse with lysosomes.

Found inside the cytoplasm of eukaryotic cells, lysosomes are membrane-bound organelles that are filled with enzymes. The main purposes of lysosomes inside eukaryotic cells are to digest nutrients inside the cell and to break down cellular debris.

Macropinosome maturation is a more complex process than endocytosis, but nonetheless, very similar. Macropinosome maturation starts by extracellular fluid being engulfed from outside of the cell membrane by the macropinosome. Then, the fluid from inside the macropinosome is reduced, which then allows for the plasma membrane proteins that was engulfed, but not needed to be recycled off of the macropinosome. With mobility, the macropinosome will then fuse with a lysosome that has already been fused with an endosome. The complex that is created by the lysosome, macropinosome and the endosome is called a endo-lysosome. Over time, the enzymes from inside this complex degrade the endosome and the macropinosome, resulting in a single lysosome (Morris, 2013).

In this study, we tested the hypothesis that mercury increases the rate at which macropinosomes mature into lysosomes inside the glial cells of a ten-day-old (*Gallus gallus*) chick embryo. We tested this hypothesis by exposing the cells, to a 100 nM solution of HgCl_2 and recorded the cells’ behaviors using a video phase microscope. The cells that were dissected from embryo were the sympathetic nerve chains.

Mercury is all around us in the environment and even a small quantity of mercury can be detrimental to human health. Three of the main effects mercury has on a human are: the disruption of the nervous system, damage to major parts of the brain and impairing their functions and damage to a person’s DNA and chromosomes (Lenntech, 2012). Knowing all of these facts about mercury made me curious as to what effects mercury had on other important organelles in the body of eukaryotic cells and if some organelles still functioned properly or “died” in the cell.

In this study, lysosomes are going to be portrayed as a darker color while macropinosomes are going to be represented by a lighter color.

Materials And Methods:

Dissection –

Ten-day old chick embryos were dissected for sympathetic nerve chains and dorsal root ganglia. Once the neurons were collected, they were placed in a petri dish filled with growth medium to grow inside an incubator at a temperature of 37 degrees Celsius (Morris, 2013 A). The materials needed for the chick dissection can be located in (Morris, 2013 A)'s lab.

Preparing For Observations –

Following the steps presented by (Morris, 2013 B), a flow chamber for the control neurons was made using growth medium as the base solution. Once the flow chamber was assembled, the neurons were cautiously transported into the ICUC room in the Wheaton College Mars Science Center for further observations.

Observations –

Using a Nikon EFD3 digital interface DFW X-700 microscope at the Apple computer labeled C-95, the neurons were observed using a 40X objective lens. A heater and thermometer was placed near the control neurons and the temperature was regulated anywhere from 32 degrees Celsius to 37 degrees Celsius.

Gathering Data –

Using the BTV application on the C-95 Apple computer, images of the neuron were captured every fifteen seconds for a total of ten minutes. After the first trial for the control neurons was completed, the second trial for the experimental neurons was assembled. 0.5 mL of 100nm solution of HgCl₂ was flowed through the flow chamber making sure no growth medium remained underneath the microscope slide. Again, every fifteen seconds for ten minutes, an image of the neurons was captured.

Measuring Cells –

To determine the size of the glial cell measure the surface area of the section you are interested in, by using the ImageJ application on the Apple computer. In this study, a line was stenciled around the surface area of a portion of the left glial cell because it showed a high quantity of macropinosome and lysosome interacting. To provide evidence to support the hypothesis, the first image captured every minute during the experiment was gathered and graphed.

Results:

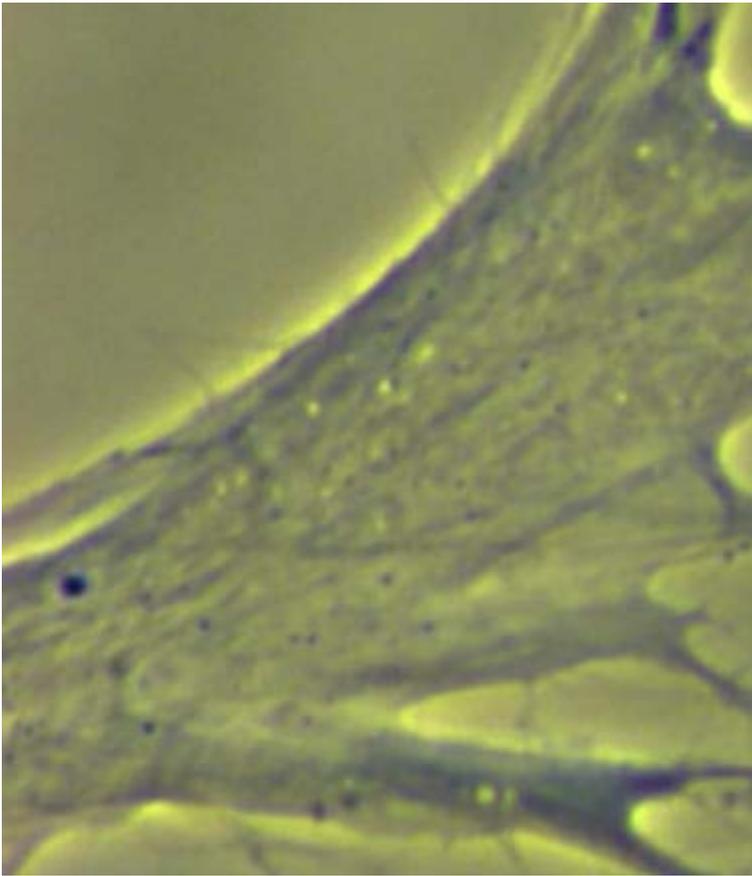


Figure 1 represents the first image from which data were derived. This image is the first image taken from the control group. The macropinosomes and lysosomes that are the most noticeable are the particles that were used to quantify the data. As shown, there are more visible lysosomes in this section of the glial cell than there are macropinosomes.

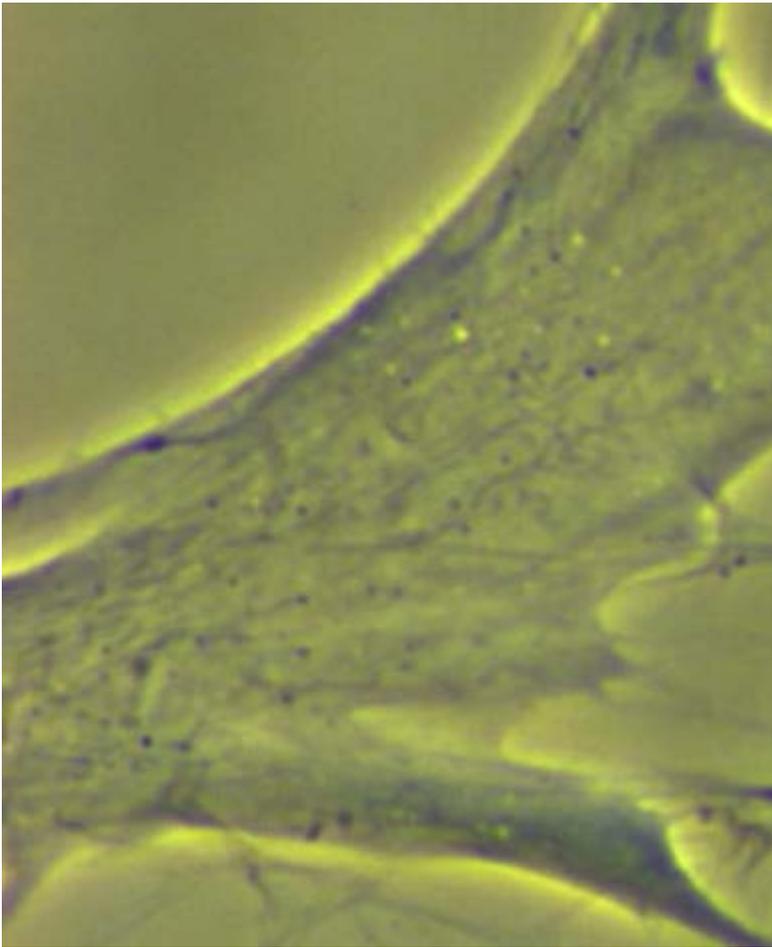


Figure 2 represents the last image in which data was concluded from. This image represents the final image of data taken from either the control group or the experiment group. In this case, this image is from the experiment group and it is apparent that there are many more macropinosomes than there are lysosomes in the glial cell.

Figure 3: Total number of macropinosomes with and without the presence of mercury

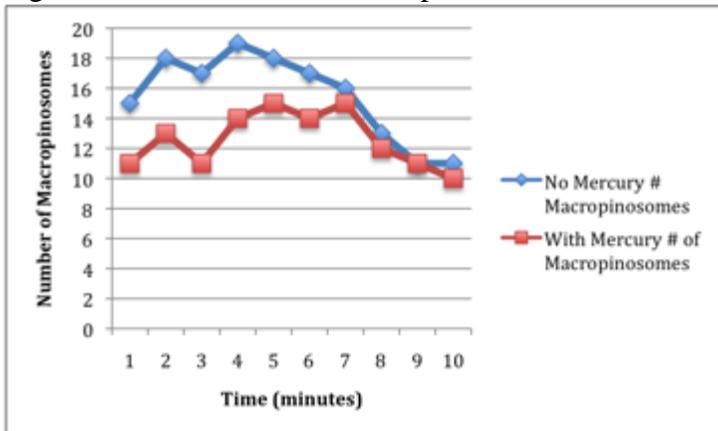


Figure 3 represents the relationship between the quantity of macropinosomes in the glial cell with and without the addition of mercury within a ten-minute time frame. Figure 3 also shows that the glial cell that does not contain mercury has more macropinosomes present inside the glial cell than the amount of macropinosomes that were present in the glial cell when mercury was added.

Figure 4: The number of lysosomes with and without the presence of mercury

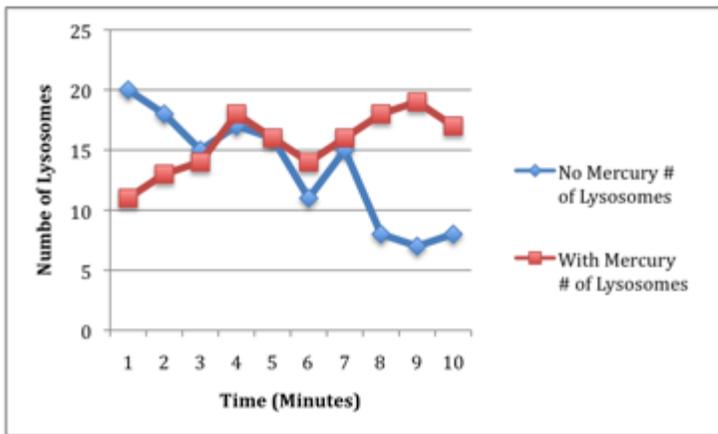


Figure 4 shows the relationship between the amount of lysosomes in the glial cell within a ten-minute time frame with and without the addition of mercury. Figure 4 also shows that on average, there were more lysosomes inside the glial cell when mercury was added than there were when mercury was not added to the glial cell.

Figure 5: Amount of particles inside the glial cell with and without the presence of mercury

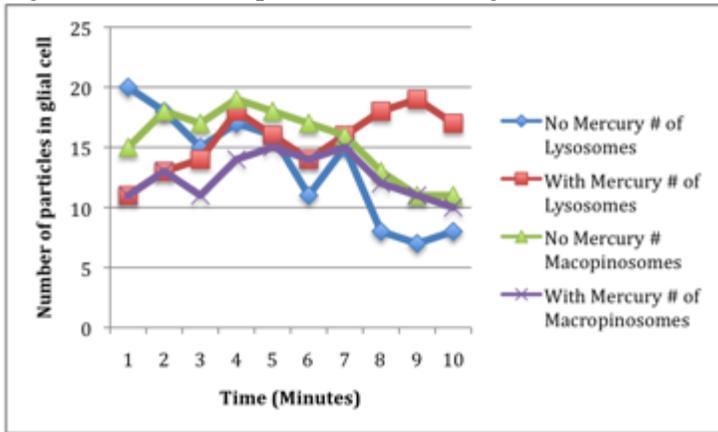


Figure 5 shows the relationship between the quantity of macropinosomes versus the amount of lysosomes inside the glial cell with and without the presence of mercury. Figure 5 demonstrates the starting and ending quantity of macropinosomes and lysosomes that were present inside the glial cell throughout the whole experiment.

Discussion:

The data from this experiment supports the hypothesis that mercury increases the maturation rate of macropinosomes to lysosomes. The surface area of the target glial cell from this experiment was 105,940 pixels. In just this surface area of the glial cell, it was very apparent that macropinosomes were maturing into lysosomes at different speeds when mercury was present and not present inside the glial cell. As figure 5 shows, the difference in quantity of lysosomes from the control to the experimental group show the most progression. This tells us, that if there were more lysosomes present while mercury had been added to the cell at the end of the ten minutes, then this should mean that macropinosomes were fusing with endosomes at a faster rate. If macropinosomes were fusing faster to endosomes then this should result in a faster bonding of the endo-lysosome complex and then the enzymes inside this complex would degrade the macropinosome / endosome faster leaving the single lysosome.

If I were to do this experiment over again, I would do two things differently. First, I would run the same types of trials, but I would extend the amount of time each trial was ran for. If the control and experiment group ran under the same heat but for longer, this would give me better data to further prove my hypothesis correct, or give me data to prove my hypothesis wrong. The second thing I would do differently, would be after the control data was collected and the experimental group was being set up, I would make sure to buffer through DMEM into the flow chamber rather than just pushing mercury into the flow chamber taking out the growth medium. The first time around, this was not

done in my experiment and I want to be sure this error did not cause the results that we saw and by doing the experiment again, I would be able to see if the same data would be gathered, or if different data would show. This would also help determine the actuality of my hypothesis.

One different experiment I would want to try, following the same protocol would be to do the same experiment, but with 4 different trials instead of two. I would want to use different doses of mercury and see if different doses of mercury would have the same effect as the 100nm solution of HgCl₂ had on the glial cell. I would use a 50nm solution of HgCl₂, the 100nm solution of HgCl₂, a 200nm solution of HgCl₂, and a control group. This would help me understand if the more mercury I used would either help speed up the macropinosome maturation even more, or if any solution higher than a 100nm solution would kill the glial cell completely. By formulating the same procedure with different levels of mercury concentrations, this would be an ideal way to determine how much mercury is detrimental to a glial cell and its macropinosomes.

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