The Observation of DNA in Glia Using Immunofluorescence to Find Mitotic Cells

Mike Koperniak
Neurobiology Short Report
Bio324 / Neurobiology
Wheaton College, Norton, Massachusetts, USA
November 12, 2014

Introduction

The brain is a fascinating organ that dictates the people we are and the lives we live. The human brain is intriguing for what it accomplishes as a circuit, but it is the roles of individual neurons and glia in the brain that can be the focus of brain malfunction and biological degradation. In brain diseases like Alzheimer's, the brain is degraded slowly over time due to malfunctioning proteins, which causes neurons and glia to eventually lose connections to surrounding cells (What is Alzheimer's, 2014)**.** Cells of the brain had been known as some of the only cells in the body that do not replicate after the developmental period until recent years when it was discovered that cells of the brain and nervous system do actually regenerate although not very frequently and typically only if there is injury to the nervous system (Gage & Temple, 2013). Unlike most cells that are constantly undergoing the cell cycle of around twenty-four hours (Cooper, 2000), neural cells do not typically undergo the cell cycle unless damage has been made to a certain region of the nervous system (Gage, 2013). In the brain, regeneration is possible through stem cells and neural progenitors. This type of study has been recently done on damaged retinal cells of *Danio rerio* (Lenkowski et al, 2014). The progenitors can signal to nearby neurons and glia, which allows them to undergo the cell cycle and proliferate but they do not cause the dead cells themselves to divide (Gage et al, 2013) (Lenkowski et al, 2014). So in theory, if scientists were able to divide cells in the brain via mitosis in patients with diseases like Alzheimer's, it could be possible to restore parts of the brain that have been lost from neurodegenerative diseases and form new synapses with new nerve cells in place of the old cells. Stem cell studies are at the forefront of biological research because there is so little known about how they could potentially help diseased patients in this manner (Tanna et al, 2014).

In this experiment, mitotic glia were tested for by dissecting 10-day old developed *Gallus gallus* embryos for their dorsal root ganglia and sympathetic nerve chains in order to grow the neurons and glia further in culture. The hypothesis was that the glia in the primary cultures would be mitotic. The way mitotic glia were found was by using immunofluorescence imaging to observe the locations and density of tubulin and the brightness of the DNA in the cells in culture. Brighter DNA meant that the DNA was more condensed and could be the result of a cell currently undergoing mitosis or a cell that has just undergone mitosis (Cooper, 2000). This hypothesis has been tested and analyzed before and there have been mitotic glia found in culture (Ge et al, 2009). It was important that this hypothesis was tested further due to the impact that understanding nervous system regeneration could have in patients with nervous injuries (Gallaher et al, 2014). One other new topic of interest is the effect that glial proliferation has on the regeneration of neurons in dorsal root ganglia (Gallaher et al, 2014). Caroline Stanclift and I collaborated in this research. The DNA of the glia in culture were imaged and used to analyze and to find mitotic glia.

Materials and Methods

Grew cells in culture. Refer to Morris (2014a) for procedure used to dissect 10-day-old chick embryonic peripheral neurons cells and the materials used. The dissected neurons were mounted on poly-lysine and laminin treated coverslips. Next was preparation for Immunofluorescence. There were 8 total coverslips, two of which were negative controls with either formaldehyde/gluteraldehyde (F/G) as the fixative or methanol (MeOH) as the fixative. The protocol for fixation and materials used were from Morris 2014b. There were changes made to this protocol regarding fixation. Sea urchin embryos were used for positive control slides but the chick peripheral neurons and glia were used for all other slides. There were 4 total experimental slides. Two of the slides were fixed with F/G and two with methanol. One experimental and one negative control received F/G while the two others received MeOH. The two positive controls were both fixed in MeOH. The coverslips were left in incubation of -80 degrees C for a week. Next, the four non-positive control coverslips were withdrawn from incubation for rehydration using PBS-T solution from Morris (2014b) post fixation. Once all coverslips were rehydrated, they were blocked using block buffer. Sea urchin embryos were fixated and permeabilized in a 15ml tube and then mounted and rehydrated and then blocked on coverslips treated with laminin. After a week in block buffer, coverslips then proceeded to the labeling phase and this began by creating the antibody and Hoechst solutions. Refer to lab protocol for immunofluorescent staining (Morris, 2014b). The three stains used for immunofluorescence were anti-polyglutamylated tubulin at a 1:500 dilution first, tetramethyl rhodamine goat anti-mouse at a 1:200 dilution second, anti-alpha-tubulin (DM1A) at a 1:100 dilution third, and the Hoechst stain last. The coverslips sat for 10-15 minutes after the Hoechst label and were then washed three times in PBS-T. Next, the coverslips were mounted on a slide on a drop of block buffer with nail polish to seal them around the edges and then brought to the ICUC to be analyzed using the Capricorn Mac computer with a Sony DWF-X700 camera and a 1.0X camera mount through SPOT software. The objective lens used on the microscope was 40x.

The data were collected using single still frames and images were taken of each slide at locations where glial cells were prominent. After several images of each slide were taken, data were analyzed using the DNA emitting images. The images were contrast enhanced by a value of 3.75 so that the best possible brightness could be achieved and so all cell DNA could be visible enough for analysis. The brightness was analyzed by using the elliptical tool in imageJ software. The region of interest in each glial cell was as much of the DNA that could be captured with the elliptical tool without getting any of the background. Using the mean brightness of each cell minus the background noise, the true brightness was calculated.

Results

Figure 1 represents the image used for the analysis of brightness of DNA. I focused specifically on the DNA for my analysis (see figure 1). The brightness values were taken and merged into five separate brightness groups to represent the trend. (see figure 1). There was clearly an abundance of less bright glia nuclei while only two glia nuclei had relatively high brightness values or medium brightness values from 15 to 20 and one was considerably brighter than the rest at a brightness of 35 (see arrow in figure 1). There was no major difference between the two types of fixed coverslips (Methanol vs. Formaldehyde/Gluteraldehyde). The negative control did not display auto fluorescence.

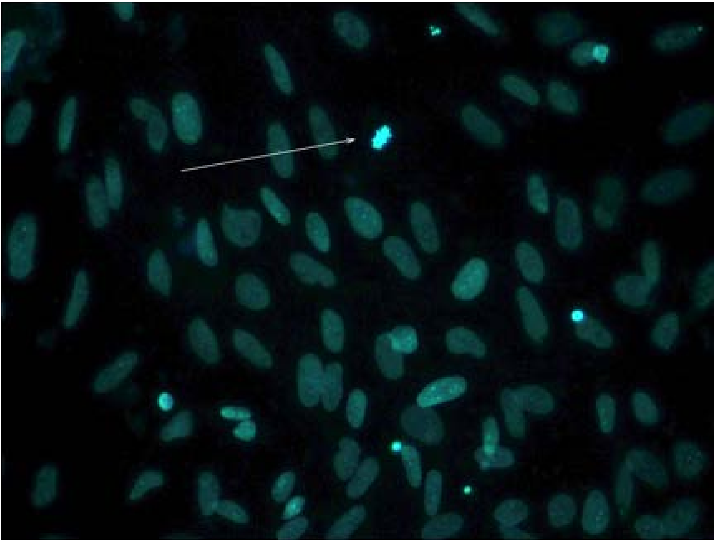

Figure 1- shows a Hoechst-stained image. The arrow points to a glial cell believed to be mitotic. Notice how bright and condensed the DNA of this glial cell is compared to the rest of the cells in the image.

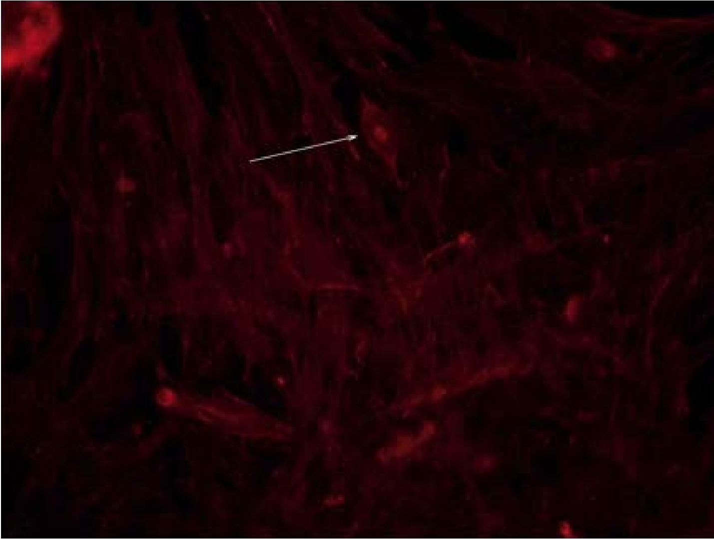

Figure 2- an anti-polyglutamylated stain image of the experimental formaldehyde/gluteraldehyde fixated slide. The arrow is pointing to a glial cell with high concentration of tubulin on the edges and middle. Note how this differs from the other cells in the image that have a uniform amount of tubulin throughout.

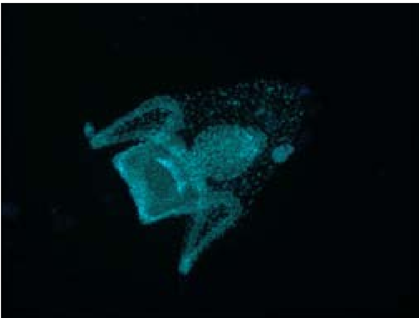

Figure 3- DNA image of the positive control sea urchin embryo. This image shows that the DNA stain, which was the basis for the data analyzed, was working correctly.

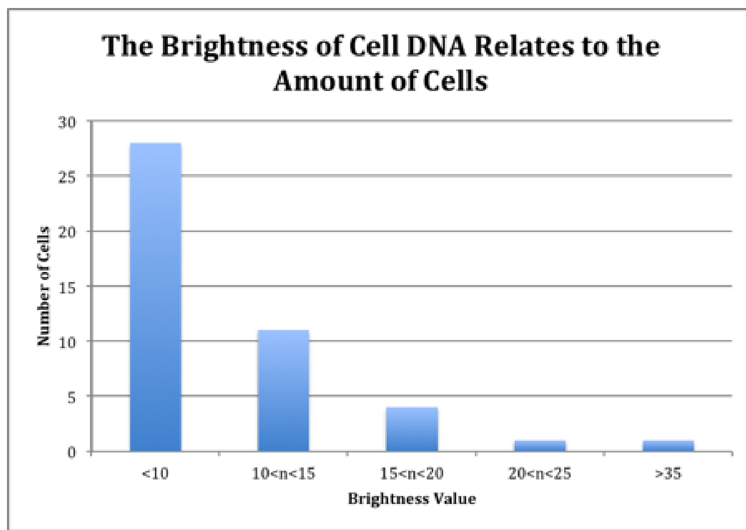

Figure 4- shows the amount of glia at each brightness value. There are twenty-eight glia under 10, 11 between 10 and 15, 4 between 15 and 20, and only one between 20 and 25 and one over 35. Notice there are much more glia with low levels of brightness while very few are at high brightness values and medium level brightness.

Discussion

Given the original hypothesis, it is likely that we have found data that shows that glial cells are in fact mitotic. These data support the original hypothesis due to evidence that was discovered in the experimental slide of what appeared to be a mitotic glial cell. The DNA of this cell was clearly condensed and undergoing the process of mitosis and when compared to the brightness values of the surrounding cells, it showed that most of the other cells had brightness values much less representing the fact that cells only condense their DNA while in a mitotic phase (Cooper, 2000). The DNA stain made a good representation of the cell cycle and it supports the idea that most cells should be in interphase while only a small percentage of cells are typically undergoing mitosis at any given time (Cooper, 2000). The percentage calculated for the data was 2% of cells undergoing mitosis.

In the experiment, error did occur but it was insignificant on the outcome and the overall data collected. The confirmation of finding a mitotic glial cell in culture leads back to the discussion of glial proliferation from Gallaher et al (2014). New evidence shows that glial cell proliferation can actually induce the proliferation of nearby neurons following a brain injury (Gallaher et al, 2014). In a future study, this hypothesis could be tested using a similar method used in this experiment but instead of just observing the glia, they could be destroyed using capsaicin and then observed for proliferation the way our study was conducted (Gallaher et al, 2014). The research on brain injuries and diseases typically begin by observing cells individually and their characteristics. If a discovery is made regarding when and where glial cells are most likely to proliferate and also how this relates to neuronal proliferation, scientists could potentially induce proliferation in the brain on patients with neurodegenerative disorders to reverse the effects of the condition (Gage et al, 2013). By confirming the discovery of mitotic glial cells in culture from dorsal root ganglia, this will hopefully allow for future experiments to be done regarding injury to cells in culture and whether or not proliferation is the response to injury. By locating the progenitors and the specific role glial cells play, proliferation in neurons could be explained better (Gallaher, 2014). Gage et al described the study of nervous system development as being one of the reasons we understand the way adult brains work and eventually led to the discovery of neural stem cells and the unlimited supply of neurons and glia that our bodies can supply us (2013). I conclude that given the data analyzed, a single glial cell in our culture was found in mitosis with bright and condensed DNA. The fact that only one mitotic glial cell was found out of the many viewed, represents the fact that the majority of cells are typically in interphase

at any given time (Cooper, 2000). We did not have a perfectly executed experiment but our data yielded a positive result. With the same experimental approach and by simply having a higher n-value, in a future experiment more mitotic glia would be found and could be used to test other hypotheses regarding glia injury and proliferation.

References

Cooper GM. The Cell: A Molecular Approach. 2nd edition. Sunderland (MA): Sinauer Associates; 2000. The Eukaryotic Cell Cycle. Available from: http://www.ncbi.nlm.nih.gov/books/NBK9876/

Gage, F., & Temple, S. (2013). Neural Stem Cells: Generating and Regenerating the Brain. *Neuron, 80*(30), 588-601.

Gallaher, Z. R., Johnston, S. T., & Czaja, K. (2014). Neural proliferation in the dorsal root ganglia of the adult rat following capsaicin-induced neuronal death. *Journal of Comparative Neurology, 522*(14), 3295-3307. Retrieved from www.scopus.com

Ge, W., Zhou, W., Luo, Q., Jan, L., & Jan, Y. (2009). Dividing glial cells maintain differentiated properties including complex morphology and functional synapses. *Proceedings of the National Academy of Sciences, 106*(1), 328-333.

Lenkowski, J. R., & Raymond, P. A. (2014). Müller glia: Stem cells for generation and regeneration of retinal neurons in teleost fish. *Progress in Retinal and Eye Research, 40*, 94-123. Retrieved from www.scopus.com

Morris R.L. (2014a). Primary Culture Of Chick Embryonic Peripheral Neurons 1: DISSECTION. Retrieved from: http://icuc.wheatoncollege.edu/bio324/2014/morris_robert/BIO324_Lab_Proc_1_Dissection_2014.htm

Morris R.L. (2014b). Immunofluor staining of SU embryos – MeOH fixation. Retrieved from: http://icuc.wheatoncollege.edu/bio324/2014/morris_robert/BIO324_Lab_Proc_4_Immunofluor_staining_of_SU_for_use_in_Neurobio.htm

Stanclift, C. (2014) Collaborator in data Collection.

Tanna, T., & Sachan, V. (n.d.). Mesenchymal Stem Cells: Potential in Treatment of Neurodegenerative Diseases. *Current Stem Cell Research & Therapy,* 513-521. Retrieved October 22, 2014.

What is Alzheimer's? (2014, January 1). Retrieved October 20, 2014, from http://www.alz.org/alzheimers_disease_what_is_alzheimers.asp