JCCC Honors Journal

Volume 7
Issue 2 *Spring 2016*

Article 3

2016

An Historical Analysis of Lab Techniques as Applies to Current Glial Cell Research

Nathan Ostermann
Johnson County Community College, nosterma@stumail.jccc.edu

An Historical Analysis of Lab Techniques as Applies to Current Glial Cell Research

Abstract
The primary focus of neurology, since the beginning, has been the neuron. This is understandable, neuronal networks and nerves being the main communication mechanism between the brain and the rest of the body. However, this focus on neurons has overshadowed and delayed research on the other cells in the brain. Glial cells, or neuroglia, are defined as all cells in the brain that are not electrically excitable neurons or vascular cells. This definition of glial cells introduced by Rudolf Ludwig Karl Virchow in 1858. Until relatively recently, the idea that glial cells are simply passive elements of the brain that only function as support for neurons dominated. Now however, scientists are discovering that glial cells do in fact have an active electrical function in the brain. In this paper, I will look at the history of glial cell research, and developments in research technology and techniques. I will analyze a 2014 publication from Mainz University in Germany, which looks at electrical communication between NG2 glial cells and neurons, to illustrate the importance of these developments for current research.

Cover Page Footnote
Kristin Thurlby, Human Sciences, served as the faculty mentor for this Honors contract.

Introduction

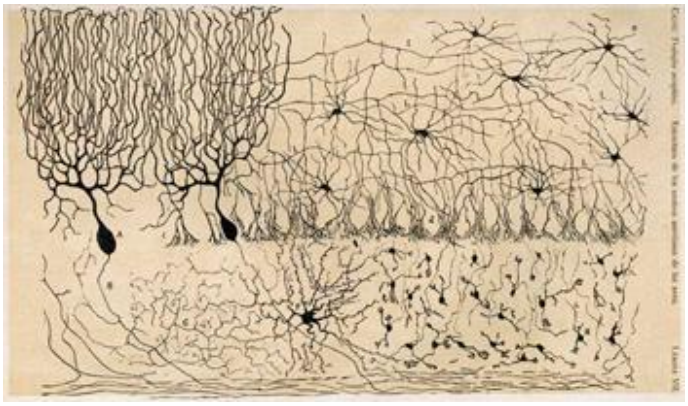
The primary focus of neurology, since the beginning, has been the neuron. This is understandable, neuronal networks and nerves being the main communication mechanism between the brain and the rest of the body. However, this focus on neurons has overshadowed and delayed research on the other cells in the brain. Glial cells, or neuroglia, are defined as all cells in the brain that are not electrically excitable neurons or vascular cells. This definition of glial cells introduced by Rudolf Ludwig Karl Virchow in 1858. Until relatively recently, the idea that glial cells are simply passive elements of the brain that only function as support for neurons dominated. Now however, scientists are discovering that glial cells do in fact have an active electrical function in the brain. In this paper, I will look at the history of glial cell research, and developments in research technology and techniques. I will analyze a 2014 publication from Mainz University in Germany, which looks at electrical communication between NG2 glial cells and neurons, to illustrate the importance of these developments for current research. [11]

History of Glial Cell Research

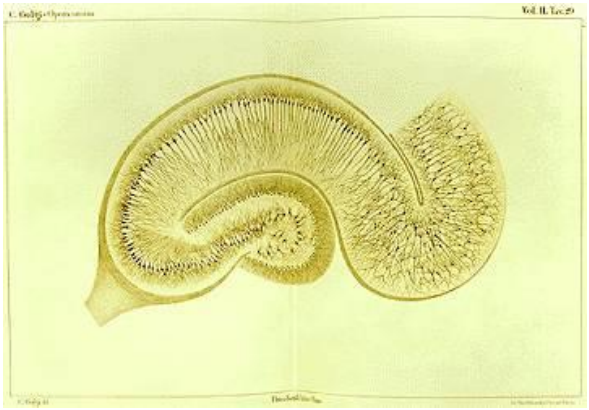
Rudolf Virchow first introduced the concept of neuroglia in a lecture he gave to a class of medical students in Germany in 1858. Virchow believed, contrary to what we known today, that glial cells were simply connective tissues that glued the

neurons together, hence the name glia, which is the Greek word for glue. Scientists made quite a bit of progress in the observation of glial cells. Much of what we know today was described, but imperfectly due to a lack of adequate technology at the time. [15]

In 1869 Jacob Henle and Friedrich Merkel made the first drawings of the neuroglial network in the grey matter of the brain. Throughout the next 80 years or so, many prominent histologists, in particular - Camillo Golgi, Gustav Retzius, Santiago Ramon y Cajal, and Pio Del Rio Hortega - led the way in glial cell research. Most of the visualizations used in neurological studies before digital technology were created by these four scientists. These early neurologists developed many of the staining techniques for observing cells. The stains they developed led to very detailed nervous system networks, cell observations, and cell classifications. Golgi and Cajal shared a Nobel Prize in Physiology and Medicine in 1906 for their work on identifying the structure of the nervous system. [15]



Ramón y Cajal's drawing of neural networks in the cerebellum.[21]



Camillo Golgi's' drawing of neural networks in the hippocampus.[22]

Several theories for neuroglial function were developed early on in neurological research. One of the two most prominent theories, suggested by Carl Weigert, was that glial cells' job was purely to fill space between neurons and give them structural support. This view was a popular view held even into the 1940s. The other popular theory, put forward by Camillo Golgi, was that glial cells' main function was to feed the neurons by controlling nutrient flow across the blood-brain barrier. Because of these views, which placed neuroglia as essentially passive elements of brain function, glial cell research was stalled and generally limited to classification. Research focused on other areas of the neuronal network. Glial cell research did not die off, however. Many neurologists had developed theories opposing those of Golgi and Weigert. [15]

Neuroscientists Carl Schleich and Ramon y Cajal theorized that glial cells, in addition to supporting and feeding the neurons, actually actively regulate synapses and neural network connections. These theories were developed in the late 1800s and early 1900s, the same time as Golgi and Weigert. After this, most of the research on glial cells was simply classification and a small amount of function was observed. Here and there something striking would be found about specific glial cells' function. For the most part however, glial cells were still regarded as passive elements. [15]

Research in neuroglial function was sparked again in the late 1950s and mid-1960s, when scientists found that there was electric coupling between glial cells. It

was not until the 1970s and 80s that two key techniques were developed for observing the electrical activities of cells at the level of individual ion channels and receptors. The use of fluorescent dyes and the whole cell recording method, along with other techniques, allowed neuroscientists to see that some neuroglia cells are actually quite active elements of the brain. [15] We will see these techniques used in the research from Mainz to show bidirectional communication between neurons and glial cells, and we will see how they really are essential for modern neurological research.

History of the Microscope

Progress in neurological research has been continually stalled by the limitations of technology. The earliest observations would have been by the naked eye or very primitive microscopes. Structures such as tissues and nerves could be observed and drawn but not in great detail compared to what we know today. This is why, up until the 1830s, scientists were only able to observe how the brain functioned on a macroscopic level. Scientists were able to observe the different lobes of the brain and which areas controlled senses, thought, and motor function, as well as the major nerve networks throughout the body.

In the early 1830s, Joseph Jackson Lister discovered a method of combining lenses that enabled greatly improved resolution from early single lensed microscopes. The new microscopes enabled far more advanced research in cellular

theory than had been possible. Lister himself was the first to observe the actual shape of mammalian red blood cells through his microscope. [3] Since then much of what we now about the anatomic structure of the brain was discovered. Cellular function, especially, in the central nervous system, was still something scientists could not completely understand.

German physicist Ernst Abbe made new forays into microscope development in the late 1870s and early 1880s. In the microscopes he developed, Abbe managed to eliminate much of the color distortions, chromatic, and spherical aberrations that had previously been in the way of getting a clear image. Abbe was partner in a microscope and optics manufacturing company. Because his developments in optics were based on mathematical precision and the physics behind optics, his company was able to effectively mass produce consistently high quality microscopes. [1] Previous microscopes relied on Lister's method of grinding lenses, which made it much harder to produce good lenses efficiently. With the new microscopes, histologists were able to observe individual cells in great detail and make very good visualizations of them.

An achromatic microscope designed by Joseph J. Lister. [24]

An early microscope designed by Ernst Abbe.[23]

In 1873 Camillo Golgi developed the silver-chromate staining method which revolutionized tissue research. Throughout the next years Golgi, and later Ramon y Cajal, developed more staining techniques that allowed histologists to identify organelles in the cells as well as their structural makeup. In 1903 Richard Zsigmondy, German chemist, invented the ultra-microscope which allowed scientists to observe specimens below the wavelength of visible light. [10]

In 1933, Max Knoll and Ernst Ruska engineered a primitive electron microscope. The electron microscope was based on the ideas of physicist Louise De Broglie that charged particles give off a certain wavelength in the light spectrum. Rather than using optical lenses, Knoll and Ruska made electrostatic fields that bounced electrons off the specimen to provide visible feedback. As this technology

was developed, the resolution and magnification of optical microscopes was soon surpassed. [7]

Development of Research Techniques

In this early phase of neurological research, the focus was on the nerves and neurons. It was commonly accepted that neuronal networks and synapses between the neurons were the principle independent method for communication between the brain and the rest of the body. Scientists saw incredibly complicated neuron networks and synapses that seemed sufficient to account for thoughts, sensations, emotions, and processing of senses such as smell, sight, and hearing. Scientists prior to the 1950s did not do extensive research on the neuroglial cells which make up at least 50% of cells in the brain.[2] Glial cells were still regarded as electrically passive cells that function to support, feed, and insulate the neurons. The glial cell classified as microglia was known to act as an immune cell, searching out and destroying harmful pathogens.

In 1961, Walther Hild published an experiment where he was able to measure glial cells' electrical activity for the first time using a microelectrode on glial cells grown in tissue culture. Hild's methods were used again in the early 1960s and the idea of glial cells having some form of actual electrophysiological impact on neuronal synapses became a possibility that scientists now began to consider and study in depth. Glial cell research still did not become a main focus of

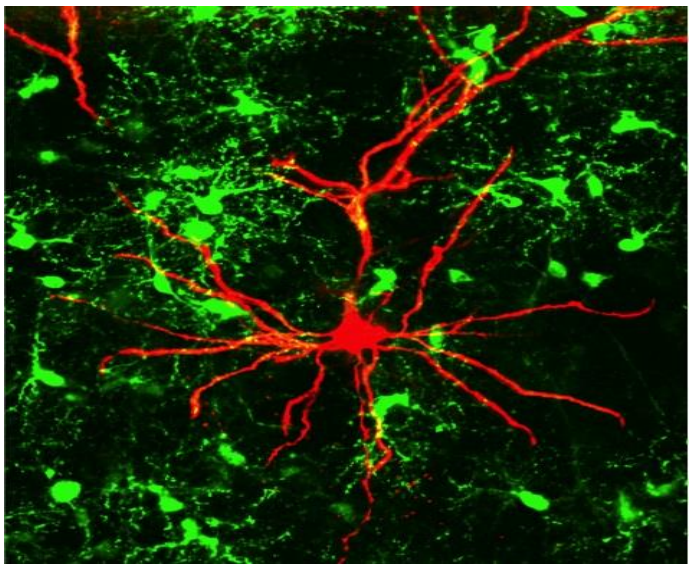
neurologists till twenty years later with the development of even more advanced techniques.

Current Research Techniques

Techniques developed in the 1970s, 80s, and 90s, have since been further refined. Scientists at Mainz University in Germany undertook to discover the mechanisms behind the electrical synapsing between one type of glial cell and neurons. An analysis of their study shows the advancements made possible by the development of technological components in research techniques.

We have known since the 50s that certain glial cells are in fact active and communicate electrically with neurons. However, we did not know how exactly, or to what effect this phenomenon happens. The cells in question, in this particular research, are oligodendrocyte precursor cells (OPCs). [11]

OPCs are cells that develop into oligodendrocytes. Approximately 5% of these OPC cells however, do not develop into oligodendrocytes. This 5% remains in close

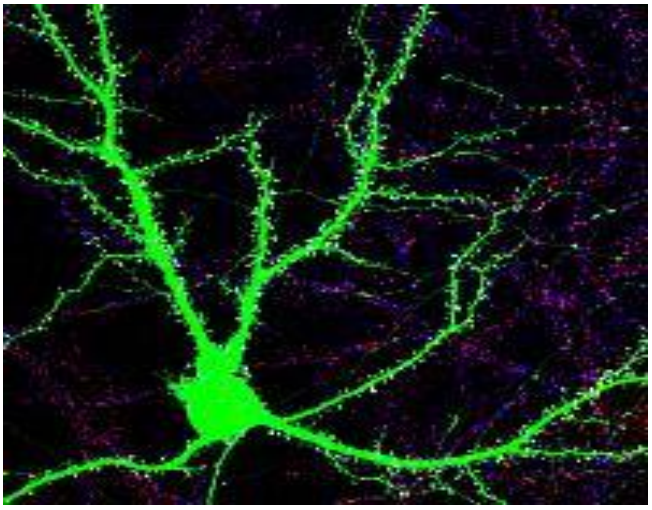


Oligodendrocyte precursor cells (green) interacting with a pyramidal neuron (red).[25]

contact with many of the neuronal synapses. OPCs are identified by their expression of nerve-glia antigen 2, or NG2. NG2 is a transmembrane proteoglycan whose traits and functions are the main focus of this research.

The scientists at Mainz found that OPC's help regulate the neuronal network through cleavage of the NG2 protein. The tests were all done on mice in this experiment. Three different types of mice were used for comparisons: wild type mice, which are simply mice that have not been genetically modified; $NG2^{-/-}$ knockout mice, which are a specially bred line of genetically modified mice whose gene for making and expressing NG2 doesn't exist; and NG2-EYFP knockin mice that express EYFP (Enhanced Yellow Fluorescent Protein) alongside NG2 in order to make the OPC cell easy to observe. 'Knockin' and 'knockout' simply refer to having a gene added or removed from the mice's genome.

EYFP is only one of many fluorescent dyes used in biological studies. The development of these dyes began in the 1960s along with the dawn of many advancements in microbiological technology and techniques. Quite a large number of fluorescent dye varieties have been developed. The first fluorescent protein was discovered in 1961 by Osamu Shimomura and Frank Johnson, who succeeded in isolating a calcium-dependent bioluminescent protein, which they named aequorin, from a jellyfish. While they were doing this they observed another protein that, when placed under an ultraviolet light, emitted a green fluorescence. The protein was named GFP (Green Fluorescent Protein). [6]



A neuron tagged with GFP.[26]

For the next twenty years scientists studied, aequorin and GFP, and how they interacted in the jellyfish. In 1992, when GFP was successfully cloned for the first time, scientists were able to use it in cell cultures outside the jellyfish. Many derivatives of GFP and similar proteins have been made since then, mostly coming from fluorescent proteins of various jellyfish and marine anemone. The dye used in this paper, EYFP is a very common dye, used quite often in neurological and cellular research.

These dyes come in two general forms: as an antibody that can bind to specific target proteins like a tag, or as a knockin gene that can be bred into a target cell and will reproduce along with that specific cell, thus tagging all target cells from the beginning of the organism being studied. The first form is sometimes known as immunofluorescence. The researchers at Mainz used both forms in their research. The immunofluorescent method was used to stain incredibly thin cortical slices of the mouse brains.

The dye would bind to the gene c-Fos. C-Fos is a gene that is often expressed when neurons fire action potentials.[20] The study found that considerably less c-Fos

was expressed in $NG2^{-/-}$ brain slices compared to $NG2^{+/+}$ slices. Fluorescent dyes have been extremely helpful in singling out specific cell lines and observing the actions of neurons and other electrically active cells. These dyes have aided advancement of neuroglial research considerably. [6]

In addition to knockout mice, the Mainz researchers also studied mouse cells that were bred in tissue culture. In tissue culture, specific cells are extracted from a parent organism and placed in an artificial environment specially conditioned for tissue growth. The first portion of the Mainz research was determining the exact mechanisms for the cleavage of NG2 in OPCs. It was found that NG2 cleavage was regulated by the enzymes ADAM10, ADAM17, and γ-secretase. ADAM10 (α-secretase), and ADAM17 are responsible for the cleavage of the NG2 ectodomain. The ectodomain is the portion of the NG2 protein that is exposed to the extracellular environment. γ-secretase is responsible for the cleavage of the intracellular domain (ICD), and is dependent on the initial ectodomain cleavage by α-secretase. These results were determined by the use of the Western blot technique and gel electrophoresis.

Western blotting, sometimes referred to as protein blotting or immunoblotting, was developed in 1979 and is being continually refined by scientists to this day. Western blotting was actually derived from the similar techniques DNA blotting and RNA blotting used to determine nucleotide sequences. Using Western blotting, scientists are able to transfer separated proteins from an electrophoresed gel onto an adsorbent membrane. [4] The gel comes from gel

electrophoresis which is the technique used to separate the proteins by molecular weight, size, and electrical charge. This is done by placing the proteins into a gel matrix and then applying an electrical current through the matrix. The proteins then move apart from one another and group into specific areas of the gel matrix depending on weight, size, and charge. [18]

The reason for the development of Western blotting techniques was that analyzing the protein characteristics with microprobes and identifying the specific proteins was very difficult while everything was still trapped in the gel. Western blotting allows for easy access to the proteins. There are a few different methods to transfer the protein from the gel to the adsorbent membrane in order to get the blot. The methods sometimes differ due to the nature of the gel or the protein. Sometimes simple diffusion can be used, however this method does not give a complete transfer. The most commonly used method for Western blotting is electroblotting. Electroblotting is faster than the simple diffusion method and gives the most complete gel-membrane transfer. It is done by either immersing the gel, sandwiched by two membranes, into a buffered transfer solution (wet transfer), or

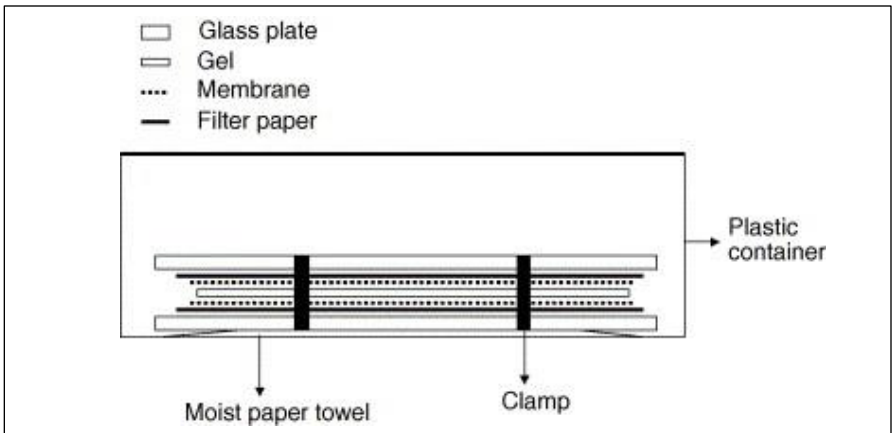


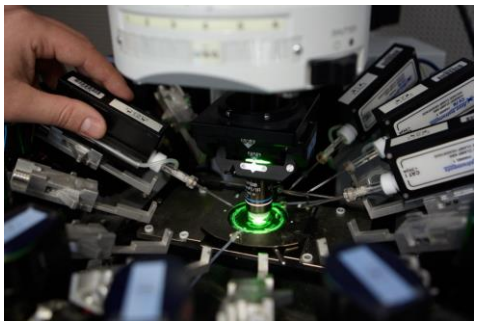
Figure 6: *Bi-directional, non-electrophoretic transfer of proteins from SDS–PAGE gels to nitrocellulose membranes to obtain up to 12 blots.* [27]

by sandwiching the gel with two buffer-soaked adsorbent membranes (semi-dry transfer). [4]

The researchers at Mainz used antibodies that tagged the ectodomain and the ICD to help make the blot visible. They showed that, when ADAM10/17 were inhibited, the number of full length NG2 proteins increased from the normal amount, this demonstrated that very little or no cleavage would take place without α-secretase.

The next step in the research was to show how the cleavage of NG2 affected long term potentiation (LTP) in the mice. LTP is a process in which increasing activity at a synapse will make long-lasting changes that enhance and strengthen the synaptic connection. In neurons of the CNS, LTP happens through the release of glutamate (one of the main neurotransmitters in the brain). Glutamate binds to AMPA (alpha-amino-3-hydroxy-5-methylisoxazole-4-propraionic acid) receptors and NMDA (N-methyl-D-aspartate) receptors. These receptors act as Ca^{++} channels that, when activated, allow calcium into the cell creating the action potential for the next synapse. In addition to this, the post synaptic cell integrates more glutaminergic receptors (NMDA and AMPA) into the post synaptic membrane, and releases a paracrine that binds to the presynaptic cell telling it to increase glutamate release. [12] The goal was to see if the NG2 ectodomain had a similar effect on the OPC regulated neuronal synapses. One of the key techniques the researchers used in discovering this was whole cell recordings.

Whole cell recording is a lab technique used in electrophysiology that enables scientists to observe ion channels and the electrical activity in individual or multiple cells. Erwin Neher and Bert Sakmann developed the method in the 1970s. Neher and Sakmann are both prominent German scientists who have studied in the USA and England. They were jointly awarded the 1991 Nobel Prize in Biology and Medicine for their development of the whole cell recording method at the Max Plank institute in Germany. [13] The technique allows scientists to take readings of electrical currents that are incredibly small (a picoampere or 10^{-12}amperes). The readings can be taken from a single ion channel in the membrane of a cell. This technique allowed Neher and Sakmann to observe exactly how the ion channels in cells function, how their shape changes and how that corresponds to electrical changes within the cell.



A patch clamp setup.[28]

The procedure is to, under a microscope, take a micropipette that is specially made to form a strong seal with the cell membrane, and insert it into a tissue sample until a seal has been made with the target cell membrane. The micropipette that was developed for this procedure was a glass pipette that was extremely thin (a thousandth of a millimeter in diameter). This was key to isolating single ion channels and seeing exactly how the channel regulated ion flow. A small amount of suction has to be applied through the micropipette in order to maintain the seal

with the cell membrane. The electrical current is recorded through microelectrodes inserted into the cell and attached to ground terminals and then a current is injected into the solution in the form of ions flowing through the pipette. This enables the maintenance of a constant voltage while measuring current. [17] The current in the ICF (intra-cellular fluid) is compared directly to that in the micropipette. Neher and Sakmanns contribution gave huge insight into several prominent diseases that are related directly to ion channel regulation such as Cystic Fibrosis, Epilepsy, and even some cardio-vascular diseases. [13]

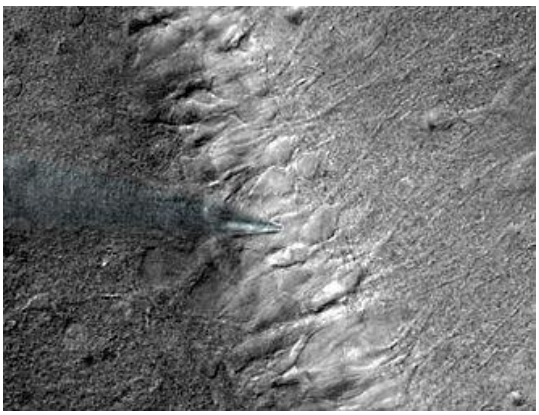


Figure 1: *Patch clamp of a nerve cell within a slice of brain tissue. The pipette in the photograph has been marked with a slight blue color.*[29]

In the OPC research from Mainz, whole cell recordings were made for the AMPA and NMDA receptors on the pyramidal neurons of the somatosensory cortex. The researchers studied the effects of electrical activity in these cells while the different receptors and proteins involved with OPC/neuron communication were inhibited separately. It was discovered that, while the presynaptic glutamate release was unaffected in $NG2^{-/-}$ mice, the composition of the AMPA receptors in the postsynaptic membrane was changed, impairing the strength of the postsynaptic current.

The last portion of the Mainz research was behavioral studies done on both $NG2^{-/-}$ and wild type mice. These tests covered the somatosensory cortex, the motor cortex, sensory motor responses, and maze tests. From the behavioral studies, no statistically significant differences were found between $NG2^{-/-}$ and wild type mice except in the sensorimotor-gating responses, which is impaired in $NG2^{-/-}$ mice.

This study reinforces other recent studies showing the ability of certain glial cells to communicate electrochemically with neurons. In this particular research, scientists discovered the details of how the glial/neuron communication actually works. It was found that OPC cells can regulate neuronal synapses and receive synapses directly from their associated neurons. The regulation takes place through the cleavage of NG2 by α-secretase and, subsequently, γ-secretase. The process was shown to be dependent on synaptic activity between the presynaptic neuron and the OPCs. The researchers found that AMPA receptors had altered properties in the somatosensory cortex in the $NG2^{-/-}$ mice. The $NG2^{-/-}$ mice were found to have significantly reduced PPI (prepulse inhibition). Prepulse inhibition is where a reaction or response is inhibited by a weaker 'prepulse' which lessens the startle response in that organism. [19] Impaired PPI has been connected to some diseases such as schizophrenia, obsessive-compulsive disorder, and Tourette syndrome. The scientists at Mainz also found that in the $NG2^{-/-}$ mice, when NG2 ectodomains were added, the impaired AMPA receptors on the postsynaptic membrane were fixed and the currents restored.

Conclusion

These results from the Mainz research are not Nobel Prize worthy, however they are a great example of the new and exciting research being done in the field of neurology. Technology has progressed to a point where we can actually study the intricacies of the central nervous system in tremendous detail. The development of the techniques used in this research has been extremely important for all the biological sciences. Fluorescent dyes, whole cell recordings, gel electrophoresis, and Western blotting have been essential to many recent discoveries made in biological studies. Scientists making new forays into neurological research are finding more and more that what we thought we knew about brain function was not completely correct, and that there is so much more to study. Small breakthroughs like this one in Germany are happening all over the research world. They are showing us that, although an important and primary structure in the brain, neural function cannot be adequately explained without better understanding the role of glial cells play in the brain. I hope to see a greater emphasis in neuroglial research in the future, and look forward to seeing what discoveries lie in store.

Works Cited

[1] Ernst Abbe. Encyclopædia Britannica. Encyclopædia Britannica Online. Encyclopædia Britannica Inc., 2016. Web. 01 Apr. 2016 <http://www.britannica.com/biography/Ernst-Abbe>.

[2] Jabr, Ferris. "Know Your Neurons: What is the Ratio of Glia to Neurons in the Brain". Scientific American. Jun 13. 2012. Web. 24 Apr. 2016. http://blogs.scientificamerican.com/brainwaves/know-your-neurons-what-is-the-ratio-of-glia-to-neurons-in-the-brain/

[3] Joseph Jackson Lister. Encyclopædia Britannica. Encyclopædia Britannica Online. Encyclopædia Britannica Inc., 2016. Web. 01 Apr. 2016 <http://www.britannica.com/biography/Joseph-Jackson-Lister>.

[4] Kurien, B. Scofield, H. "Western Blotting". Methods. Vol 38. Issue 4. 283-293. Apr 2006. PDF. http://ac.els-cdn.com/S1046202306000065/1-s2.0-S1046202306000065-main.pdf?_tid=04f47ca4-fd92-11e5-993b-00000aab0f02&acdnat=1460124109_43290db933c3ba41dfd4c0a17d3d472f

[5] Lackie, J. M. A Dictionary of Biomedicine. Oxford: Oxford University Press, 2010. Web.

[6] MicroscopyU. Introduction to Fluorescent Proteins. 2015. Web http://www.microscopyu.com/articles/livecellimaging/fpintro.html

[7] Microscope. Encyclopædia Britannica. Encyclopædia Britannica Online. Encyclopædia Britannica Inc., 2016. Web. 01 Apr. 2016 <http://www.britannica.com/technology/microscope/The-theory-of-image-formation>.

[8] Okubo, Y. and Iino, M. (2011), Visualization of glutamate as a volume transmitter. The Journal of Physiology, 589: 481–488. doi:10.1113/jphysiol.2010.199539

[9] Paredes, R. Madelaine et al. “Chemical Calcium Indicators.” Methods (San Diego, Calif.) 46.3 (2008): 143–151. PMC. Web. 31 Mar. 2016.

[10] Richard Zsigmondy. Encyclopædia Britannica. Encyclopædia Britannica Online. Encyclopædia Britannica Inc., 2016. Web. 01 Apr. 2016 <http://www.britannica.com/biography/Richard-Zsigmondy>.

[11] Sakry D, Neitz A, Singh J, Frischknecht R, Marongiu D, Binamé F, et al. (2014) Oligodendrocyte Precursor Cells Modulate the Neuronal Network by Activity-Dependent Ectodomain Cleavage of Glial NG2. PLoS Biol 12(11): e1001993. doi:10.1371/journal.pbio.1001993

[12] Silverthorn, Dee U. Human Physiology: An Integrated Approach. San Francisco: Pearson/Benjamin Cummings, 2007. Print.

[13] The Nobel Prize in Physiology or Medicine 1991. Nobelprize.org. Nobel Media AB 2014. Web. 29 Mar 2016. http://www.nobelprize.org/nobel_prizes/medicine/laureates/1991/press.html

[14] The Nobel Prize in Physiology or Medicine 1906. Nobelprize.org. Nobel Media AB 2014. Web. 30 Mar 2016. http://www.nobelprize.org/nobel_prizes/medicine/laureates/1906/

[15] Verkhratsky, Alexei, and Butt, Arthur Morgan, eds. Glial Physiology and Pathophysiology. Somerset, GB: Wiley-Blackwell, 2013. ProQuest ebrary. Web. 22 March 2016.

[16] Wardell, W. M. "Electrical and Pharmacological Properties of Mammalian Neuroglial Cells in Tissue-Culture." Proceedings of the Royal Society of London. Series B, Biological Sciences 165.1000 (1966): 326-61. Web.

[17] Wikipedia contributors. "Patch clamp." Wikipedia, The Free Encyclopedia. Wikipedia, The Free Encyclopedia, 21 Feb. 2016. Web. 30 Mar. 2016.

[18] Wikipedia contributors. "Gel electrophoresis." Wikipedia, The Free Encyclopedia. Wikipedia, The Free Encyclopedia, 7 Apr. 2016. Web. 9 Apr. 2016.

[19] Wikipedia contributors. "Prepulse inhibition." Wikipedia, The Free Encyclopedia. Wikipedia, The Free Encyclopedia, 10 Feb. 2016. Web. 25 Apr. 2016

[20] Wikipedia contributors. "C-Fos." Wikipedia, The Free Encyclopedia. Wikipedia, The Free Encyclopedia, 7 Mar. 2016. Web. 25 Apr. 2016

Image References

[21] Wikipedia contributors. "Santiago Ramón y Cajal." *Wikipedia, The Free Encyclopedia*. Wikipedia, The Free Encyclopedia, 5 May. 2016. Web. 15 May. 2016.

[22] The Nobel Prize in Physiology or Medicine 1906. *Life and Discoveries of Camillo Golgi.* Nobelprize.org. Nobel Media AB 2014. Web. 15 May 2016. https://www.nobelprize.org/nobel_prizes/medicine/laureates/1906/golgi-article.html

[23] Page direct from google images. 15, May 2016. https://www.pinterest.com/pin/391320655092645344/

[24] Page direct from google images. 15, May 2016. https://commons.wikimedia.org/wiki/File:An_achromatic_microscope_designed_by_Joseph_J._Lister_Wellcome_M0010915.jpg#file

[25] Sakry D, Neitz A, Singh J, Frischknecht R, Marongiu D, Binamé F, et al. (2014) Oligodendrocyte Precursor Cells Modulate the Neuronal Network by Activity-Dependent Ectodomain Cleavage of Glial NG2. PLoS Biol 12(11): e1001993. doi:10.1371/journal.pbio.1001993

[26] Buttery, Philip. University of Cambridge. *Dr. Philip Buttery.* Web. 15, May 2016. http://www.neuroscience.cam.ac.uk/directory/profile.php?pcb10

[27] Kurien, B. Scofield, H. “Western Blotting”. Methods. Vol 38. Issue 4. 283-293. Apr 2006. PDF. http://ac.els-cdn.com/S1046202306000065/1-s2.0-S1046202306000065-main.pdf?_tid=04f47ca4-fd92-11e5-993b-00000aab0f02&acdnat=1460124109_43290db933c3ba41dfd4c0a17d3d472f

[28] Blue Brain Project. *Blue Brain Project.* Web. 15, May 2016. http://www.artificialbrains.com/blue-brain-project

[29] Wikipedia contributors. "Patch clamp." Wikipedia, The Free Encyclopedia. Wikipedia, The Free Encyclopedia, 21 Feb. 2016. Web. 30 Mar. 2016.