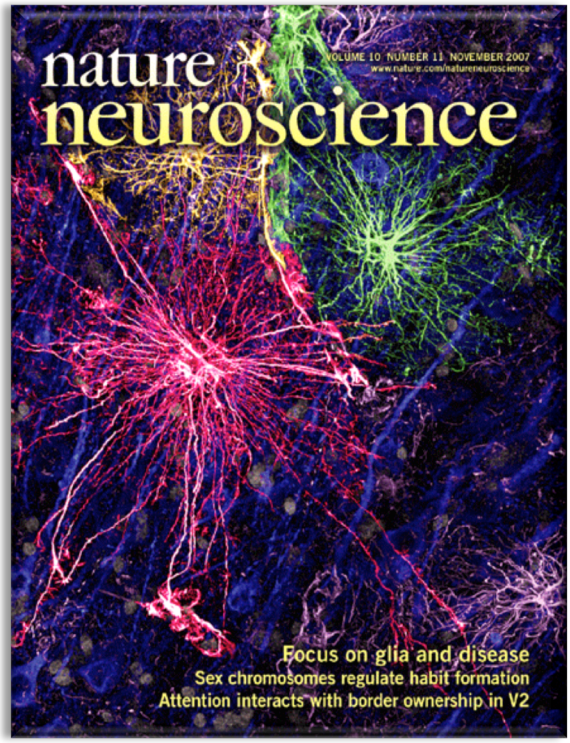
Neuroglia: An Investigation Into Evolving Perspectives



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Abstract:

Historically neurons have dominated the center stage in neuroscientific research as the primary nervous system cells that propagate electrical signals, and thereby underpin physiological and behavioral manifestations. However, our currently advancing technology has allowed researchers to expand their scope of inquiry. Over the last 30 years, groundbreaking research has been conducted in the area of glial science. Neuroscientists today are building upon the foundation poured by the pioneers of yesterday to elucidate the excitingly distinct functions of neuroglia. No longer regarded as simple, passively supportive cells, current research indicates that neuroglia are highly active cells, which contribute to a number of neural processes including synaptic plasticity, neurogenesis and neurodegeneration. This paper presents an in-depth look at neuroglia, including an historical look back at the primary research of yesterday, and how these events shaped the evolution of glial science through to today. Fundamental morphology and function for each glial sub-type within the central and peripheral nervous systems is also presented. Utilizing this knowledge as the backdrop to the most current findings in glial science, this paper discusses the unique contributions that glia make in neurological health and degradation, particularly in regards to neurodegenerative diseases, including Alzheimer's.

Introduction

In the field of neuroscience, neurons have dominated the center stage of interest as the sole propagators of action potentials, and thereby regulators of behavior (Jacobsen, 1993). However, in both the central and peripheral nervous systems, the cells of the so-called 'other brain' also reside: neuroglia (Fields, 2009). Steeped in the shadow of the neuron's electrical capacity, these cells have been largely ignored since their discovery in the 1850's. However, our advancing technology is finally ushering neuroglia out of the darkness as revolutionary discoveries in glial physiology have redefined the traditional functional perspective of glia (Malva, et al., 2007).

The term 'neuroglia' describes a number of determined sub-classes of cells that reside in both the central and peripheral nervous systems (Kettenman & Ransom, 2005). In the central nervous system, astrocytes and oligodendrocytes are the primary macroglia. Microglia compose the second central nervous system sub-class of neuroglia. In the peripheral nervous system, Schwann cells are the only glial cells (de Velis, 2002). Each of these subclasses will be discussed in depth later.

In the human nervous system, glial cells outnumber neurons by a factor of about 10. Since the methods for staining and visualizing the various subtypes of glia have not been perfected, the exact ratio has not yet been precisely determined (Verkhratsky & Butt, 2007). However, it is estimated that *Homo sapiens* have about 85 billion glial cells, not counting the neuroglia of the cerebellum, which have been estimated to number around 105 billion (Kettenmann & Ransom, 2005).

It is important to note that the study of glia is not a recent development in the scientific community. What scientists know today about the evolutionary origin, histology and physiology of the variety of neuroglia in the nervous system is a product of decades of research (Kettenman & Ransom, 2005). While the discoveries of today, assisted by modern technology, are certainly incredible, the stories of yesterday's scientific inquiries are highly important, not to mention incredibly entertaining.

Chapter 1: Historical Overview

In considering the discovery and ensuing studies of neuroglia, Kettenmann and Ransom make an excellent observation in stating that the scientific discoveries of the time were essentially by-products of the enveloping 'intellectual milieu' (2005). Of primary importance to consider is the emergence of the cell theory as first proposed by botanist Carl Ludwig Schleiden. Theodor Schwann would apply the concept of the cell theory to his work on animal tissue in 1839 following a dinner conversation with Schleiden (Kettenmann & Ransom, 2005).

The work of these and other renown pioneers including Robert Remak, Rudolph Albert Kölliker, Jakob Henle and Rudolf Virchow, would coalesce to provide the foundation for what Jacobosen (1993) notes is "the most important generalization in the science of morphology, viz., the principle of structural similarity in animal and vegetable tissues (23)." The cell theory and its constituent precept *omnis cellula e cellula*, cells arise only from other cells, would spread to

influence the research of all cytologists, histologists, and physiologists of the 19th century (Kettenmann & Butt, 2005).

The concept that the nervous system may contain both active, or excitable cells, as well as passive, or non-excitable cells, was first introduced by Gabriel Gustav Valentin in 1836 (Kettenmann & Butt, 2005). However, the study of the non-excitable cells would not be marked until nearly a decade later. In 1846, Rudolf Virchow commented in his paper *On the Granulated Appearance of the Ventricular Walls*, "The connective substance forms a sort of cement in the brain, spinal cord and higher sensory nerves, in which the nervous elements are embedded" (Kettenmann & Ransom, 2005). From this quotation, it is apparent that Virchow began the study of glia not by recognizing the histological markers of individual cells, but by describing the space between neurons. Thus, the beginnings of glia are rooted in the supposition that they were a ubiquitous mass of connective tissue, not individually functioning cells (Jacobsen, 1993).

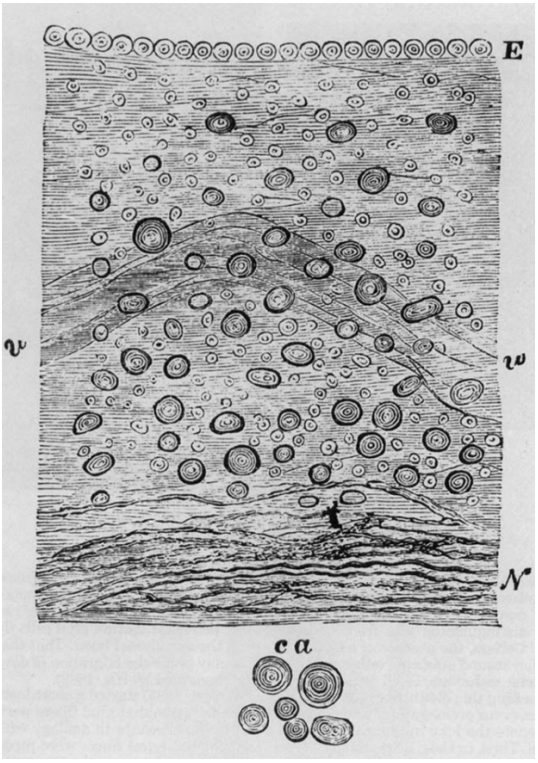

Figure: One of Virchow's original drawings of what are now known as neuroglia. (brown.edu)

The term neuroglia was not coined until 1856, by none other than Virchow himself (Kettenmann & Ransom, 2005). He derived the word from the Greek root word Υλια, meaning something slimy or sticky. Interestingly, this root now

translates to mean something filthy, or someone of debased morality (Verkhrastsky & Butt, 2007)

Virchow's 1858 publication *Cellular Pathology*, contained his first illustrations of what he deemed 'neuroglia': "…the real cement, which binds the nervous elements together, and that in all its properties it constitutes a tissue different from the other forms of connective tissue, has induced me to give it a new name, that of neuro-glia" (Kettenmann & Ransom, 2005). Throughout his publication, Virchow used the term *nervenkitt*, or 'neural putty', interchangeably with neuroglia (Verkhratsky & Butt, 2007).

It is important to note that Virchow was not actually illustrating separate cells in this work, but was using the term neuroglia as a marker for the interstitial substance that we now know to be the processes of macroglial cells, astrocytes in particular (Ranson, 2003). Eventually, as studies progressed in conjunction with staining techniques, the term neuroglia became synonymous with neuroglial cells. Virchow recognized that the interstitial substance contained cellular elements, but he simply referred to them as 'connective tissue corpuscles' or 'elements of the neuroglia', rather than defining them as distinctive cells (Kettenmann & Ransom, 2005).

While Virchow is most often given the credit for the 'discovery' of glial cells, Heinrich Müller actually noted in 1851 that the retina contained radial fibers (Mori, et al., 2005). One year later, Rudolf Kölliker would name these Müller fibers, as they are still known today. Also, Müller published an illustration of much better

clarity and precision than Virchow in 1858, the same year as Virchow's praised publication. Max Schultze followed one year later in 1859 with even more refined illustrations (Kettenmann & Ransom, 2005).

Although other scientists may have produced work prior to Virchow, Jacobsen explains that Virchow's "theory led and the facts followed" (Jacobsen, 1993). Regardless, the incredible thing to note is that all of these scientists conducted their early studies without stain, having to tease apart individual cells with fine needles! This explains why most of the pioneering research conducted in this area was done on the retina rather than the brain, and why Müller glia were the most well-described glial cell of the time (Kettenmann & Ransom, 2005).

With the advent of improved staining techniques, histological studies of neuroglia improved and further distinctions of neuroglial sub-types were made (Verkhratsky & Butt, 2007). Santiago Ramón y Cajal heralded Otto Deiters as the scientist responsible for the discovery of stellate cells in white and gray matter (Cajal, 1984). Today these cells are known as astrocytes, a term synonymous at the time with stellate cells, Deiter's cells & arachnoid cells (Cajal, 1984).

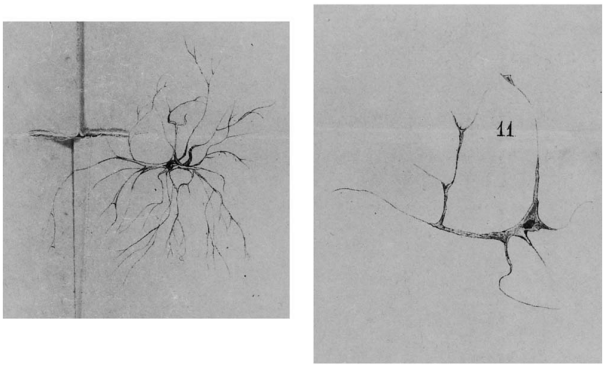

Figure: Illustrations of stellate glia by Otto Deiters. (brown.edu)

In 1869, Jakob Henle would expand upon Deiter's initial research to produce the first illustrations of the cellular networks formed by stellate cells in the spinal cord (Verkhratsky & Butt, 2007).

Yet, the modern term 'astrocyte' was not coined until 1893 by Michael von Lenhossek in the first extensive review of glial cells in a textbook, focusing primarily on the spinal cord. Von Lenhossek is credited with recognizing that the term neuroglia should be used *cum grano salis*, with a grain of salt, because in reality it referred to multiple types of cells. After its inception, the term astrocyte was used variably. Andreizen & Kölliker used von Lenhossek's premise as a springboard, further dividing astrocytes into fibrous and protoplasmic subtypes (Kettenmann & Ransom, 2005).

Camillo Golgi also conducted glial research, which was reflected in his 1870 & 1871 papers outlining his staining techniques using hematoxylin and carmine. Notably, he observed the endfeet of astrocytes contacting blood vessels, which would later provide evidence for his nutritional theory (Verkhrasky & Butt, 2007). Golgi also recognized that the molecular layer of the cerebellum contained glial fibers. While these fibers were originally discovered in 1857 by Karl Bergmann, the cells still carry the name Golgi epithelial cells, as well as Bergmann glia (Mori, et al., 2007).

Heralded as his greatest contribution to neuroscience, Golgi's *reaziona nera*, the dichromate silver stain, also spurred further classification of glia. Although it did not distinguish neurons from glia, Carl Weigert indicated that it facilitated

study regardless: "One recognized neuroglia as the structure that one could not or would not call neuronal" (Kettenmann & Ransom, 2005). However, it was the honing of this stain by Andriezen and Cajal that led to the elucidation of the fine structure of both neurons and glia.

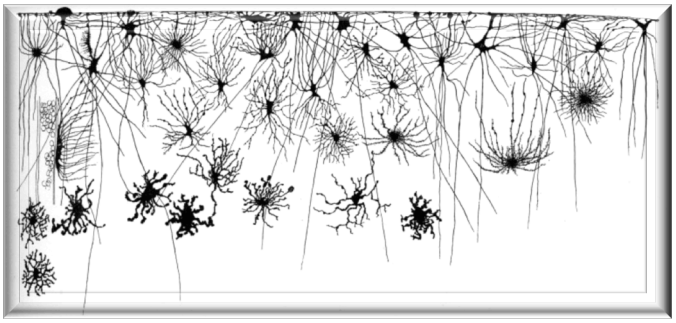

Figure: Golgi-stained glial cells of a human frontal cortex. (Matyash & Kettemann, 2009)

In fact, in 1909 Cajal stated that, "We have emphasized that two special elements which are really abstractions derived from many examples, form all neural tissue: the neuron with its various processes and the glial cell" (Kettenmann & Ransom, 2005).

A second theory that von Lenhossek supported was the notion that glia formed a neurospongium, or syncytium. While an unsupported theory due to technological limitations, this notion held until the 1950's when the advent of the electron microscope was able to provide definitely opposing proof otherwise (Verkhratsky & Butt, 2007).

Along a similar line, the study of myelin, a term originated by Virchow in 1858, and associated glia suffered due to inefficient technology. In 1838, Robert Remak technically produced the first illustration of a glial cell; an illustration that is recognized now as a Schwann cell. The term Schwann cell was not used until 1871, as coined by Louis-Antoine Ranvier. Remak recognized the myelin sheath surrounding peripheral never fibers, deeming them *tubulus primitivus*. These

observations and his recording of bundles of unmyelinated axons, later termed Remak fibers, was confirmed by Theodor Schwann in 1839 (Kettenmann & Ransom, 2005).

Based on these observations, Schwann proposed the *catenary*, or cell-chain theory, whereby nerve fibers yield an aggregation of continuous nuclei forming a chain of cells (Verkratsky & Butt, 2007). Unfortunately, this misguided notion held until the 20th century with some of the most influential scientists supporting it. Cajal was quoted as saying, "Many writers, including ourselves, view myelin itself as nothing more than a secretory product of axons, rather than the contents of a cell that the axon passes through" (Kettenmann & Ransom, 2005).

Until the early 20th century, Schwann cells and astrocytes dominated the histological aspect of neuroglia. However, in 1913 Cajal introduced the gold chloride-sublimate stain (Kettenmann & Ransom, 2005). Again, this technique stained astrocytes particularly well, but other glia were now also visible. Cajal deemed these 'other cells' the third element of the central nervous system and continued on with his neuronal advocacy. It was Cajal's pupil, Pio del Rio-Hortega, who would delve into this third element to elucidate its constituents (Kettenmann & Ransom, 2005).

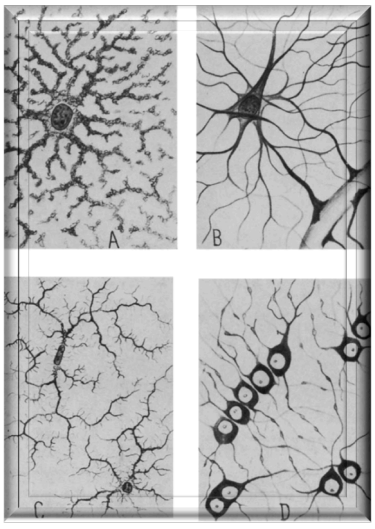

Figure: Del Rio-Hortega's four types of glia: protoplasmic, fibrous, microglia & oligodendrocytes (brown.edu)

Rio-Hortega's defining moment came with the development of the silver carbonate stain and the

unveiling of oligodendroglia and microglia, terms that he coined himself. Recognizing that oligodendroglia were more prominent in white matter, Rio-Hortega proposed that these glia were classical, meaning of ectodermal origin, as well as the producers of myelin. Interestingly, Cajal, having failed to replicate his student's work, and also partial to the notion that myelin is simply a product of axons, distrusted Rio-Hortega and his theories, thereby destroying their once close relationship (Kettenmann & Ransom, 2005).

Rio-Hortega also noted that microglia are distinct from other glia as they are of mesodermal origin, with migratory and phagocytic properties, much akin to blood macrophages. To Rio-Hortega, "…the microglia constitutes the true third element of the central nervous system" (Kettenmann & Ransom, 2005). Using his specific silver carbonate stain, Rio-Hortega was also able to recognize the difference between ramified and ameboid forms of microglia, with only the ameboid form displaying the macrophage-like abilities. Jacobsen notes, "In historical perspective that what Cajal is to the neuron, Rio-Hortega is to the neuroglia" (Jacobson, 1993).

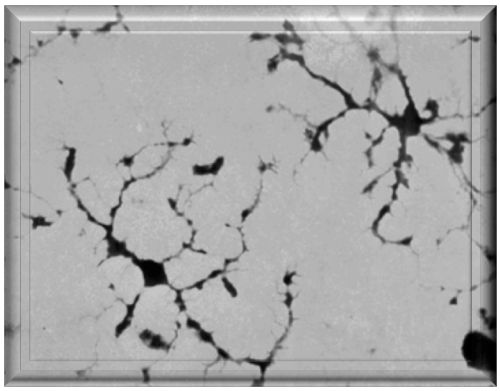

Figure: Silver stained microglia. (missinglink.ucsf.edu)

Due to the limited techniques of the time, scientists of the late 19th and early 20th century relied primarily on staining methods and low-resolution microscopes to discern the complexity of the human nervous system. Unfortunately, these provide

relatively ineffective methods to study function (Mori, et al., 2007). However, several theories remained prominent.

Santiago Ramón y Cajal, as the eminent father of neuroscience, was the primary consultant for the consideration of theory (Kettenmann & Ransom, 2005). In his immense *Histology of the Nervous System*, which focuses almost entirely on astrocytes as he did not consider myelin a property of glia, nor did he trust Rio-Hortega's work, Cajal proposes the leading functional theories of glia. For example, he refuted Golgi's proposition that astrocytes, due to their contact with capillaries, carried nutrients to neurons (Cajal, 1984). Cajal's opinion prevailed through the 1960's and has only recently been wholly overturned in the 1990's (Scemes & Spray, 2012).

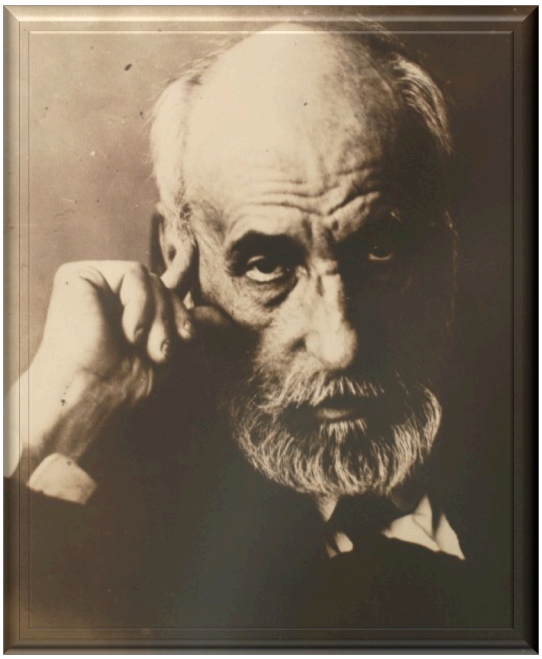

Figure: Santiago Ramón y Cajal (cnrf.org)

In conjunction, Cajal strongly opposed Weigert's *filling theory*, which proposed that neuroglia play an entirely passive role in the nervous system, simply filling-up space between the neurons. Unfortunately, despite Cajal's refutation, this theory maintains a presence even today. Finally, Cajal also comments on his brother Pedro's isolation theory. Pedro Cajal proposed that glia serve as insulators to neuronal impulses (Cajal, 1984). Today, while this theory is known not to be

entirely wrong, at the time Santiago Cajal did not agree with the proposed theory of myelin as a glial-derived insulator (Verkhratsky & Butt, 2007).

When considering the initial theories surrounding the primary function of neuroglia, it is important to mention Carl Ludwig Schleich, a student of Virchow (Verkhratsky & Butt, 2007). In his 1894 publication *Schmerzlose Operationen*, Schleich was the first to propose active neuronal-glial interactions, whereby neurons and glia were equal players in nervous system functioning. Schleich believed that glia were the inhibitory element of the brain as controlled by the constantly shifting volume of the cells. For example, swollen glia around a neuronal connection would prevent the propagation of neuronal communication and vice versa (Kettenmann & Ransom, 2005). Unfortunately, Schleich's proposition was presented the same year that Sigmund Exner pronounced the Neuron Doctrine. With enormous support from scientists like Cajal, the Neuron Doctrine stomped out any interest in pursuing a role for glia in the limelight of nervous system function (Verkhratsky & Butt, 2007).

From its inception in the early 20th century through to today, the Neuron Doctrine has held its throne in neuroscientific research. The neuron remained the most studied cell in the brain, with glia cast in the shadows as passive 'glue.' However, with the advent of the electron microscope in the 1950's the darkness began to ebb for neuroglia. Improved methods for staining, processing and sectioning tissue, as well as freeze fracturing and quantitative image analysis

methods have allowed researchers to expand their scope of inquiry (Webster & Åström, 2009).

The tide began to irrevocably turn for glia with two discoveries in the 1960's. First, in 1966 Steven Kuffler, Richard Orkand, and John Nicollis discovered that there is an electrical coupling between glia (Kettenmann & Ransom, 2005). Second, in 1969, Miltorn Brightman and Tom Reese identified the gap junctions that allowed glia to communicate with each other (Verkhratsky & Butt, 2007). While these two research teams initiated the renewed interest in glia, neuroglia were still regarded as passive for two more decades, providing only nutritive and supportive help to the neurons (Kettenmann & Ransom, 2005).

Finally, in 1984 Helmut Kettenmann and Harold Kimelberg made the groundbreaking discovery that cultured astrocytes and oligodendrocytes expressed glutamate and GABA receptors. Not long after, in 1990, Ann Cornell-Bell and Steve Finkbeiner found that astrocytes are capable of long-distance communication via propagating calcium waves. In addition, they determined that these calcium signals are stimulated by the neurotransmitter receptors found in the glial membranes (Kettenmann & Ransom, 2005).

With these pioneering discoveries fueling research endeavors over the last twenty years, further inquiries have demonstrated that glial cells, astrocytes in particular, express nearly every type of neurotransmitter receptor currently known, as well as a plethora of ion channels that are stimulated by both intracellular and extracellular events (Wang & Bordey, 2008). In short, this indicates that glial cells

can actually detect the activity of neighboring neurons. In addition, neuroglia are capable of sending feedback signals not only to other glia via gap junctions, but also to neurons via secretion of neurotransmitters, including glutamate and ATP over chemical synapses (Jourdain, et al., 2007). In general, today's research demonstrates that glia are capable of not only sensing their microenvironment and that of neighboring neurons, but they also have the ability to modulate these conditions via chemical and electrical signaling amongst themselves, and with neurons, to respond adequately (Verkhratsky & Butt, 2007).

The revival of glial science that is slowly blossoming today is a product of the groundbreaking discoveries of the 1960's. Since then, each new analysis of glia has pushed the boundaries of the Neuron Doctrine to bring about a renewed interest in neuroglia (Kettenmann & Ransom, 2005). In the last twenty years alone, scientists have made tremendous strides in clearly defining not only the supportive roles of glia, but also their incredibly novel functions, which not only facilitate neuronal health, but also disease and degradation (Verkhratsky & Butt, 2007). To understand the physiological complexity of glia, it is first important to assess their evolutionary history and general histology.

Chapter 2: Histology

Glia are not unique to our species, or even to vertebrates for that matter. Seen relatively early on in the evolutionary scheme, neuroglia also reside in the primitive nervous system of invertebrates, including cephalopods and insects. In

fact, extensive study of these animals has allowed researchers to learn much about glial morphology and function (Verkhratsky & Butt, 2007).

As the evolutionary tree continues to branch its fine processes towards complexity, the central nervous systems of more multifarious organisms are characterized by an exceptional increase in both the number and the diversity of glial cells. For example, primate nervous tissue exhibits some types of glia that are much larger and more complex than those of a non-primate. Also, the human central nervous system contains specific subclasses of glia that are not present in other organisms, including interlaminar and polarized astrocytes. This is in sharp contrast to the rather fixed shape and dimension of neurons, as well as the stable density of their synaptic contacts over the evolutionary spectrum (Oberheim, et al., 2006).

The function and morphology of glia depend on the specific location of the cell and its resident microenvironemt. In the central nervous system there appear to be three types of glia, which encompass several sub-classes under a single umbrella term. These include astrocytes and oligodendrocytes. Microglia also reside in the central nervous system, and are a unique subclass all their own. Outside of the brain and spinal cord, Schwann cells are the primary peripheral nervous system glia (Verkhratsky & Butt, 2007). The specific physiology of each type of glia will be discussed in depth later. It is important to note that there are also satellite glia within the sensory and sympathetic ganglia, as well as in the enteric nervous system, but these will not be examined in detail (Kettenmann & Ransom, 2005).

Table 1 below briefly summarizes the major classes of neuroglia that will be discussed.

Table 1: Primary Divisions of Neuroglia

Types of Neuroglia	Location	Primary Function
Astrocytes	CNS	Highly Varied
Oligodendrocytes	CNS	Myelination in the CNS
Schwann Cells	PNS	Myelination in the PNS
Microglia	CNS	Immunocompentent Cells of the CNS

Like neurons, differentiated macroglia develop from the primary germ cell layer known as the ectoderm. During early development of the neural tube, neuroepithelium cells, also known as pluripotent neural stem cells (NSC's), give rise to oligopotent neural progenitors. These progenitor cells then differentiate a limited number of times to produce either neuroblasts or glioblasts, the precursor cells for neurons and glia, respectively (Taupin, 2006).

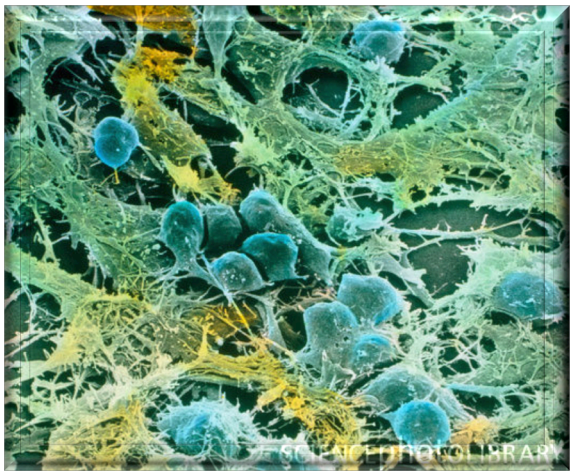

Figure: SEM of neuroglia; immature (blue), mature (green) (sciencephotolibrary)

Classical theory held that glioblast and neuroblast lines were distinctly separate and unrelated. However, as glial science has continued to blossom, recent research has procured evidence indicating that products of

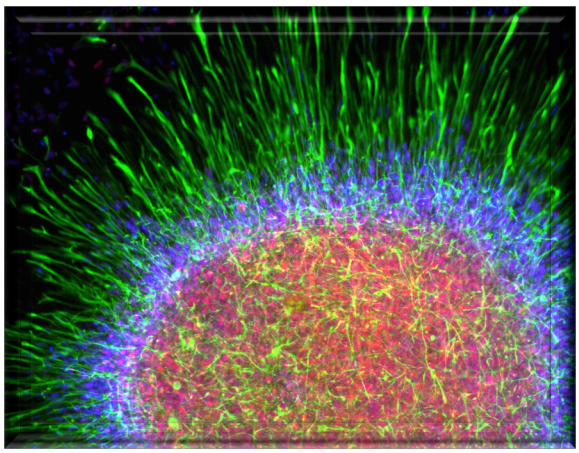

Figure: Astrocyte progenitors & immature astrocytes aggregate to form "astrospheres." (Robert Krencik/ UW-Madison)

glioblasts may actually be multipotent neural precursors. This means that radial glia maintain the ability to produce not only other glia, namely other types of astrocytes, but neurons as well. Also, it appears that radial glia, and their astrocyte descendants, maintain their stem cell-like properties throughout life (Taupin, 2006).

While the origin and lineage of each sub-class of neuroglia are certainly important in discerning how glia function within the nervous system, a cornerstone to understanding the specific subclasses of glia continues with an overview of their general histology and morphology. In considering the peripheral nervous system, the distinct characteristics of Schwann cells will be presented, in addition to a discussion of the neuroglia of the central nervous system, including astrocytes, oligodendrocytes and microglia.

Astrocytes

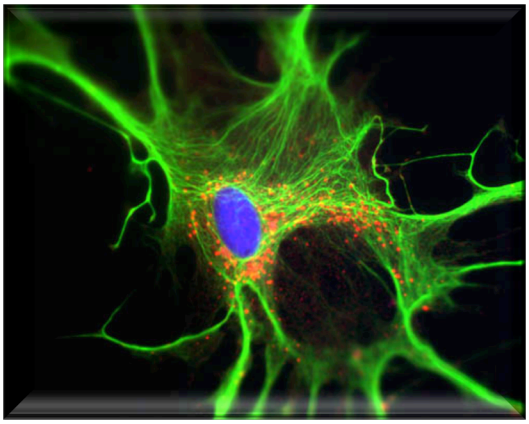

Figure: 'True' astrocyte (www.saasta.ac.za)

The label astrocyte is an inclusive term meaning 'star-like'. The most abundant and diverse type of cell within the nervous system, astrocytes range in size, shape and function depending on their location within the central nervous system (Kettenmann & Ransom, 2005). However, it has been established that the expression of glial fibrillary acidic protein and vimentin is common to all types of astrocytes. Deviating from this characteristic,

the dimension of astrocytic function and morphology exist on a spectrum (Sofroniew & Vinters, 2010).

Radial glia are bipolar cells with oblong cell bodies and elongated processes (Verkhratsky & Butt, 2007). These processes have endfeet that connect to the ventricular wall or pial surface, crossing both white and gray matter. As neural progenitors, radial glia are the first cells to develop, and typically disappear from most regions of the brain as they develop into stellate astrocytes throughout the perinatal period. However, two sub-classes of radial glia have been identified post-maturation, including Müller cells of the retina, and Bergmann cells of the cerebellum (Mori, et al., 2005).

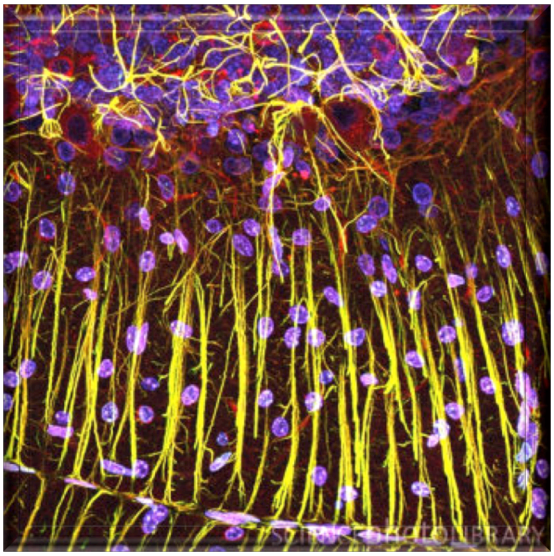

Figure: Confocal light micrograph of cerebellum tissue: Purkinje cells (red), radial glia (yellow), nuclei (purple) (sciencephotolibrary)

Müller glia have processes that run along the rods and cones of the eye, composing nearly 20 percent of the retina's volume. With a ratio of about 1 to 16, Müller cells make contact with distinct neurons in a columnar manner (Mori, et al., 2005).

Bergmann glia, or Golgi epithelial cells, usually display between three to six processes, which surround several Purkinje cells and terminate near the pia mater. The dendritic arborization of their processes form what appears to be a 'tunnel'

around the neighboring neurons. Around 8,000 synapses are covered by a single Bergmann cell (Wang & Bordey, 2008).

Protoplasmic and fibrous astrocytes, deemed 'true' astrocytes, display classical stellate morphology.

Protoplasmic astroglia are found in gray matter, with particularly high concentrations in the hippocampus. Their morphology exhibits many elaborate and fine processes, which end in perivascular feet, contacting blood vessels. Other processes extend to the pial surface. As the most numerous astrocyte, protoplasmic astroglia account for the greatest surface area of cortex (Sofroniew & Vinters, 2010). In fact, a single protoplasmic astrocyte envelops around two million neuronal synapses (Verkhratsky & Butt, 2007).

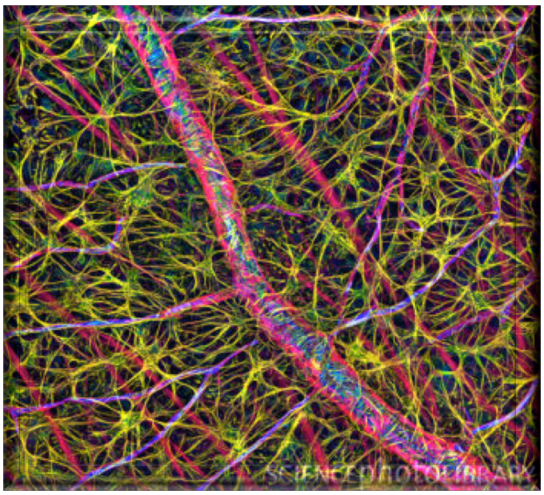

Figure: Fluorescent light micrograph of astrocytes (yellow) contacting blood vessels (red/blue) (sciencephotolibrary)

In contrast, fibrous astrocytes are found in the central nervous system white matter. These cells exhibit long, simple processes, which either end in perivascular, subpial or perinodal feet. The density of fibrous astrocytes has been measured to be 200,000 cells per mm^3 (Verkhratsky & Butt, 2007). Each of these astrocytes maintains a distinct territory (Kettenmann & Ransom, 2005).

Most current research has centered on these so-called 'true' astrocytes and radial glia (Bachoo, et al., 2004). However, there also exist a number of other

astrocytic sub-types localized in the central nervous system. A few of these sub-classes will be given a general overview. Yet, many of these sub-types remain relatively unexamined, and thereby will not be discussed in detail.

Tanycytes are the most common macroglial cell found in the nervous tissue of lower vertebrates (Verkhratsky & Butt, 2007). In humans they are located in periventricular organs, the pituitary and the raphe of the spinal cord. These cells form tight junctions with capillaries to help solidify the blood-brain-barrier and form a permeability layer between neural tissue and the cerebral spinal fluid. Serving a similar function, perivascular and marginal astrocytes close to the pia mater form endfeet with blood vessels to preserve the glia limitans. This 'limiting membrane' separates neural tissue from the vascular tissues of the pia and the subarachnoid space. There is no contact between these astrocytes and neurons (Verkhratsky & Butt, 2007).

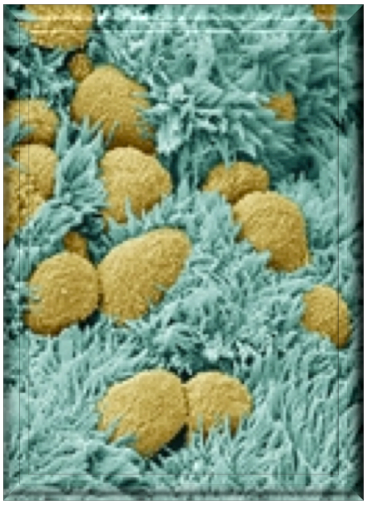

Figure: SEM of ependymal cells (yellow) & cilia of the ventricular space. (sciencephotolibrary)

Considered distinct macroglial cells, ependymoctyes and choroid plexus cells line the ventricular space. Together, these cells secrete cerebral spinal fluid and facilitate its movement through the use of microvilli and kinocilia (Verkhratsky & Butt, 2007).

Along a similar design are retinal pigment epithelial cells, which line the sub-retinal space. These cells also form a blood-cerebrospinal fluid

barrier. They are responsible for the resorption of water within the sub-retinal space. A characteristic feature of these RPE cells is the exhibition of a melanin pigment assumed to restrict the scattering of light (Verkhratsky & Butt, 2007). Interestingly, it is also suggested that these granules may be, as Kettenmann and Ransom suggest, "sinks for free radicals and excited oxygen species" (2005). Table 2 below summarizes the variety of astrocytes discussed with their respective location and functions.

Table 2: Sub-types of Astrocytes

Name	Location	Primary Function
Protoplasmic ("True")	Grey Matter	Highly Varied
Fibrous ("True")	White Matter	Highly Varied
Bergmann (Radial)	Cerebellum	Perception of Synaptic Function
Müller	Retina	Perception of Synaptic Function
Velate	Cerebellum	Insulation
Interlaminar	Supragranular Layer to Cortical Layer V	Unknown (specific to CNS of higher primates)
Tanycytes	Periventricular Organs, Hypophysis & Raphe of the spinal cord	Formation of the blood-brain barrier
Pituicytes	Neurohypophysis	Insulation
Perivascular & Marginal	Blood-Brain Barrier	Formation of the *glia limitans*
Ependymocytes	Lining of the Ventricular Space	Secretion & Regulation of CSF; Stem Cells

Oligodendrocytes

In contrast to the overwhelming diversity of astrocytes, oligodendrocytes maintain a more closely related morphology and function. Oligodendrocytes do not

appear to have any stem cell-like qualities as they originate from common oligodendrocyte progenitor cells, or OPC's. Their specific phenotypic differentiation is thought to be contingent upon neighboring axonal signals (Verkhratsky & Butt, 2007).

Oligodendrocytes are classified into four phenotypes, I-IV, based on their morphological manifestations, including number of processes and the size of the axons with which they make contact. Types I and II have small somas from which four to six processes extend to myelinate ten to thirty thin axons. These axons are usually less than two micrometers thick and maintain internodal lengths between 100-200 μm.

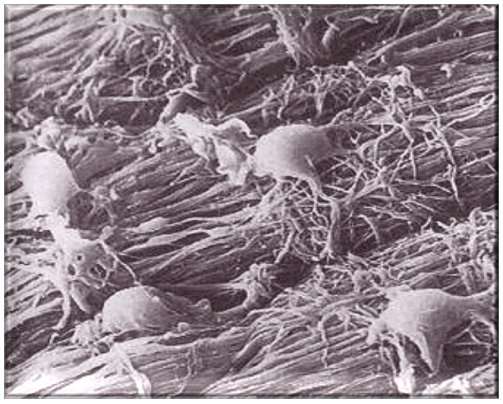

Figure: EM showing branched oligodendrocytes extending processes to axons. (regencel.com)

Type I cells are found in the cerebellum, forebrain and the spinal cord, while type II oligodendrocytes are found only in white matter, including the corpus callosum and the optic nerve (Armati & Mathey, 2010).

Type III oligodendrocytes have a larger cell body from which several thick processes extend to myelinate up to five axons that are 4-15 μm in diameter. These axons maintain an internodal length between 200-500 μm. Type III cells are located in the peduncles, medulla oblongata and the spinal cord (Armati & Mathey, 2010).

Type IV oligodendrocytes are found exclusively around the entrances of the nerve roots into the central nervous system (Verkhratsky & Butt, 2007). They have no processes and form a single myelin sheath, which extends to leave a 1000 μm internodal space along the largest axons. Besides the four phenotypes described, there are also satellite oligodendroglia, which are non-myelinating cells of the gray matter. Their specific function is unknown, but they appear to adhere to neuronal somata (Armati & Mathey, 2010).

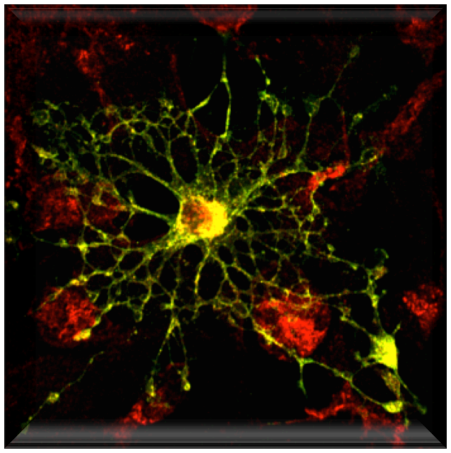

Figure: Confocal image of oligodendrocytes. (em.mpg.de)

Often associated with oligodendrocytes, NG-2 expressing glia also reside in the central nervous system. Bearing specific markers of oligodendrocyte precursor cells, and a morphology similar to that of adult astrocytes, NG-2 glia are of assumed oligodendroglial lineage (Verkhratsky & Butt, 2007). However, they do not express the exact markers of mature oligodendrocytes or astrocytes. Ongoing studies suggest that the NG-2 glia may develop into either myelinating oligodendrocytes or non-myelinating NG-2 glia, which bear a resemblance in form and function to astrocytes (Verkhratsky & Butt, 2007). Some sources indicate that NG-2 glia may compose a completely distinct type of macroglia (Kettenmann & Ransom, 2005).

With small perikarya and numerous thin, radially oriented processes, NG-2 glia are not mitotically active post-maturation, but may be induced to be such following a central nervous system insult. These cells are found in both gray and

white matter. Those in the gray matter extend processes in all directions and are in close contact with neurons, even forming functional synapses as they receive afferent neuronal signals. White matter NG-2 glia exhibit polarized processes, which extend along myelinated axons to end in perinodal feet. Interestingly, it seems that these glia form extensive contacts with not only neurons, but also astrocytes, oligodendrocytes, myelin sheaths and the glia limitans. However, they do not contact each other, maintaining a segregated territory of about 200-300 μm in diameter (Verkhratsky & Butt, 2007).

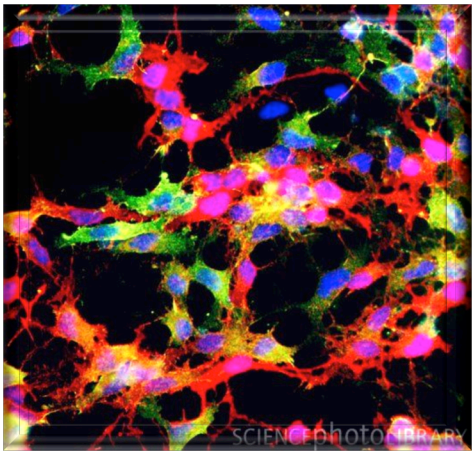

Figure: Fluorescent light micrograph of glial stem cells, stained for NG2 protein (red). (sciencephotolibrary)

Microglia

The final class of neuroglia within the central nervous system is composed of microglia. These cells are myelomonocytic in origin, stemming from the mesodermal germ layer. Fetal macrophage progenitors enter the neural tube early in development, moving towards the perinatal white matter of the corpus callosum. Here they give rise to amoeboid microglia, often described as 'fountains of microglia' (Kettenmann & Ransom, 2005). Following proliferation throughout the brain, these cells convert to their resting state known as ramified microglia, during which they are highly branched. Featuring a small soma with intensely branched, thin

processes, this is the normal state for microglia within a healthy central nervous system (Luo, et al., 2010).

However, upon insult, these immunocompetent cells become activated and morph into their amoeboid reactive state, which allows them to move through nervous tissue more easily. Microglia can also convert to a phagocytic form. Microglia of the gray matter have processes that extend in all directions, while white matter microglia processes remain perpendicular to neighboring axon bundles (Bruce-Keller, 1999).

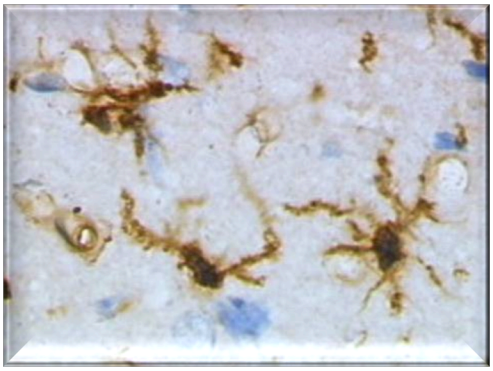

Figure: Immunohistological staining of activated microglia
(doi:10.1038/nrneurol.2010.17)

Constituting 10 percent of all glia in the central nervous system, microglia are seen in the highest concentrations in the olfactory telencephalon, the basal ganglia, the substantia nigra and the hippocampus. Each cell encompasses its own territory (Verkhratsky & Butt, 2007).

Schwann Cells

In the peripheral nervous system, Schwann cells are the primary glial components. Deriving from precursor cells of the neural crest, Schwann cells migrate along axons to their intended location based on axonal signals. It is interesting to note that these glia will die if they lose contact with an axon prior to differentiation, and yet they are also capable of de-differentiation.

There are three types of Schwann cells, which are primarily classified by their function: myelinating, non-myelinating and perisynaptic. Myelinating Schwann cells cover large diameter axons, typically over one micrometer in diameter to form nodes of Ranvier, while extending perinodal processes that communicate via gap junctions. Non-myelinating Schwann glia envelop small diameter axons by producing a membrane sheath around individual axons within a nerve fiber to form Remak bundles (Verkhratsky & Butt, 2007).

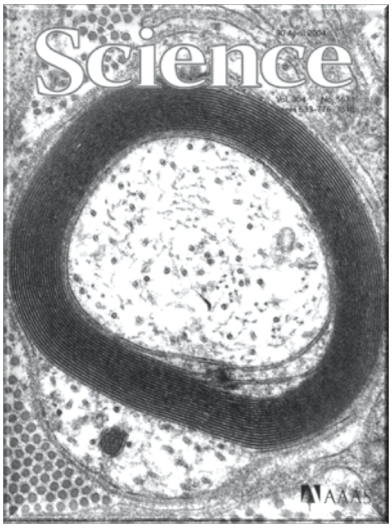

Figure: EM cross-section of myelinated axon. (sciencemag.org)

The other type of Schwann cell is composed of perisynaptic glia, found at the neuromuscular junction. Forming a sheath around terminal axon branches and synaptic boutons, these cells play a role in the maturation and development of the motor endplate (Kettenmann & Ransom, 2005).

Histology and location most often dictate function. The morphology discussed above is highly varied, contributing to each glial cell's own unique capabilities. However, there remain some anatomical characteristics that are generally ubiquitous to all sub-types.

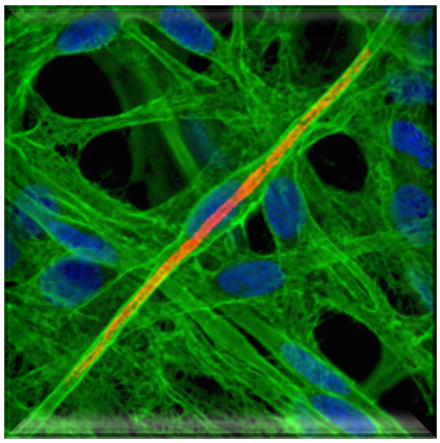

Figure: Confocal immunofluorescent image of myelinated dorsal root ganglion. (geisinger,org)

Chapter 3: General Physiology

The groundbreaking discoveries of the 1960's triggered the renewal of glial science, illustrating that glia may play an integrative role in the functioning of the nervous system (Kettenmann & Ransom, 2005). Attention then turned to the particular physiological elements that glia exhibit and how this would affect neuronal function. Thus began the quest to solidify the area of glial science through the study of glial expression of neurotransmitter receptors, ion channels and intercellular connections (Oberheim, et al., 2006).

The distribution of ions across the glial membrane and extracellular environment is akin to that of the neuronal microenvironment (De Keyser, et al., 2008). There is an elevated concentration of sodium and calcium on the outside of the cell, while potassium concentrations remain higher intracellularly. However, one important difference is that the concentration of chloride is higher within the cell. This is attributed to the increased activity of the sodium-potassium-chloride co-transporters that move two chloride ions into a cell while taking one potassium ion and one sodium ion out (Verkhratsky & Butt, 2007). The distribution of ions across the glial cell membrane will become important for a later discussion of gap junction signaling.

Research conducted over the last twenty years indicates that glial cells express all types of voltage-dependent ion channels. This includes the major ions involved in neuronal communication: sodium, potassium and chloride. Physiologically, these ion channels closely resemble the ion channels of neurons &

muscle cells (Ranson, et al., 2003). Table 1.3 provides a summary of the primary ion channels present in the glial membrane.

Table 1.3 Primary Ion Channels of the Glial Cell

Type of Ion Channel	Proposed Function
Calcium Channels	Induction of Ca2+ signals
Sodium Channels	Suggested: regulation of proliferation
Delayed Rectifier Potassium Channels	Reset of resting membrane potential; glial proliferation & reactivity
Chloride Channels	Chloride ion trasport; regulation of cell volume
Aquaporins	Water regulation

(adapted from Verkhratsky & Butt, 2007)

All types of glia have a highly negative resting membrane potential. This is due to inward rectifier potassium channels (Kettenmann & Ransom, 2005). Atypical, these voltage-dependent ion channels remain closed when the cell is depolarized, but spring open during hyperpolarization as the membrane voltage drops nearer to Ek. Favoring the inward movement of potassium, rectifier potassium channels set the resting membrane potential of glial cells between -80 mV to -90 mV (Verkhratsky & Butt, 2007).

The movement of ions across the neuroglial membrane certainly causes an electrical depolarization of the cell. However, it is an electrotonic change in membrane potential, which does not produce regenerative action potentials. This local change of ionic current results only in graded potentials (Verkhratsky & Butt, 2007).

In conjunction with the expression of similar ion channels to neurons, glial cells also demonstrate the capacity for the expression of the same multitude of neurotransmitter and neuromodulator receptors, both ionotropic and metabotropic.

This allows glial cells to sense the activity of neighboring neurons during synaptic transmission (Scemes & Spray, 2012). Astrocytes appear to have the greatest and most diverse expression of receptors, followed by oligodendrocytes, and then microglia. The distribution and specificity of these receptors is dependent on the glial cell's location within the nervous system, as well as its intended function. In other words, the receptors expressed by neuroglia are limited and complementary to the neurotransmitters released by neighboring neurons. Thus, glial cells are optimized to sense near-by synaptic activity based on their repository receptors (Verkhratsky & Butt, 2007).

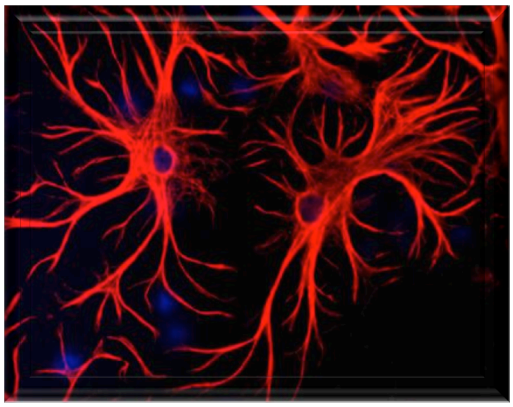

Figure: Classic, 'star-shaped' astrocytes. (www.encorbio.com)

The array of receptors expressed by neuroglia is just as vast as that expressed by neurons. It includes receptors for neuropeptides, purines, indolamines, catecholamines, endothelins, cytokines and chemokines (Sofroniew & Vinters, 2010). The scrutiny of each of these receptors is beyond the scope of this paper. However, there are a couple of interesting findings associated with a few of these receptors, which deserve mention. For example, the expression of the receptors for chemokines, cytokines and neuropeptides on astrocytes and microglia appear to be highly important in the regulation of cell growth, differentiation and pathological reaction (Hamby & Sofroniew, 2010).

On a similar note, an area of intense research is the expression of P2X purinoreceptors by microglia, specifically the P2X7 subtype. Activated by high levels of extracellular ATP, these receptors result in a massive influx of cations and the possible release of biologically active substances from microglia. This suggests that the P2X7 receptors enable microglia to sense neuronal damage and respond appropriately. Researchers postulate that these receptors are highly important for the activation of microglia and the manifestation of their ensuing macrophage properties. For a more in-depth analysis of glial receptors, please see Verkhratsky & Butt's 2007 edition of *Glial Neurobiology*, chapter five.

A fascinating characteristic of glia, beyond their ability to sense synaptic transmission, is their capacity to communicate amongst themselves via gap junctions and hemichannels. Through direct intercellular contact, neuroglia are able to form connections with the same subtype, homocellular gap junctions, and across subtypes, heterocellular gap junctions (Barger & Van Eldik 1992). The coordination of electrical and metabolic activity of groups of cells, as well as the amplification of signal transduction, are the primary functions of the glial syncytium (Kettenmann & Ransom, 2005).

Astrocytes display the greatest degree of coupling, most often homocellularly, with an average pair of cells being connected by about 230 gap junctions. Histological studies using Lucifer Yellow have shown that the staining of a single astrocyte reverberates to about 50-100 neighboring cells. Interestingly, it has also been shown that astrocytes maintain the capability to form gap junctions with

neurons, especially during early development. However, the degree of coupling and integration varies by cortical location (Verkhratsky & Butt, 2007).

Oligodendroglia also form connections to one another via gap junctions, but they are much weaker than those of astrocytes.

They are also capable of forming gap junctions with astrocytes to compose the "panglial syncytium", which is suggested to play a general integrating role, extending even to ependymal cells (Verkhratsky & Butt, 2007)a. In contrast, microglia and NG-2 glia do not couple either to one another or to other glial subtypes. It is hypothesized that this allows these glia to act on an individual basis in response to brain injury and pathology Bruce-Keller, 1999).

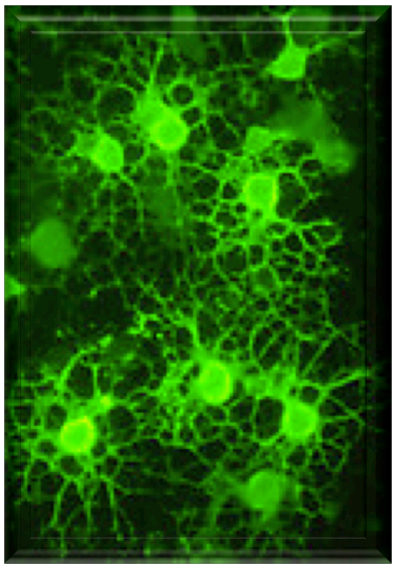

Figure: Fluorescent light micrograph of cortical mice oligodendrocytes. (sciencephotolibrary)

The ability of neuroglia to contact each other via gap junctions has provoked an intense study of Ca2+ homeostasis and signaling within glia. While neuroglia maintain all of the typical 'machinery' necessary for Ca2+ storage, expression and regulation, there remains one important deviation; neuroglia have a very limited number of voltage-gated Ca2+ channels. In fact, mature macroglia do not express these channels at all as the propagation of calcium currents is down-regulated through maturation, suggesting that these currents may play a role in development and differentiation (Verkhratsky & Butt, 2007).

In mature glia, the transport of calcium is achieved through a variety of ligand-gated channels and store-operated channels, with the main source of calcium signaling stemming from the endoplasmic reticulum where the intra-ER Ca2+ concentration is lower than that of neurons. The rapid release of calcium from the endoplasmic reticulum follows the metabotropic activation of inositol triphosphate, which binds to receptors on the endoplasmic reticulum. This causes the opening of the store-operated Ca2+ channels, which are responsible for the production of Ca2+ signals that outlast the initial stimulus and vary in temporal and frequency coding (Verkhratsky & Butt, 2007).

The mechanism behind this activation remains relatively unclear, but it is known that the reciprocity of Ca2+ release, ER reuptake and entry shapes the signal. As Verkhratsky & Butt (2007) indicate, "Intracellular propagation of Ca2+ signals is determined by a special property of the ER membrane, which, similar to the plasmalemma of excitable cells, is able to convert a local supra-threshold response into a propagating wave of excitation (69)."

In other words, the propagation of prolonged calcium waves is an indication of glial excitability (Bruce-Keller, 1999). This appears to be a unique property of astrocytes, which includes the intercellular diffusion of inositol triphosphate through gap junctions and the triggering of a metabotropic receptor-mediated Ca2+ release in neighboring cells via the regenerative release of an extracellular messenger such as ATP. Once propagated, a calcium wave can travel at about 15-20 µm/s for 300-400 µm, allowing astrocytes to communicate over long distances.

The notion of a passive, non-excitable astrocyte has certainly been debunked. While the substrate may be different from neurons, the wave propagation of the ER membrane causing the slow opening/closing of Ca2+ channels, the implication remains that glia are indeed excitable cells, which are capable of sensing neuronal activity (Verkhratsky & Butt, 2007).

Yet, the extent of glial interaction at the synaptic level does not stop with basic sensation. Instead, it has been shown in the last twenty years that glia, particularly astrocytes, actually release neurotransmitters, including glutamate and GABA (Herts, et al., 1999). While it has been demonstrated that oligodendroglia, microglia and NG-2 glia respond to neurotransmitters released by neurons and astrocytes, it has not yet been proven that they release neurotransmitters themselves (Verkhratsky & Butt, 2007).

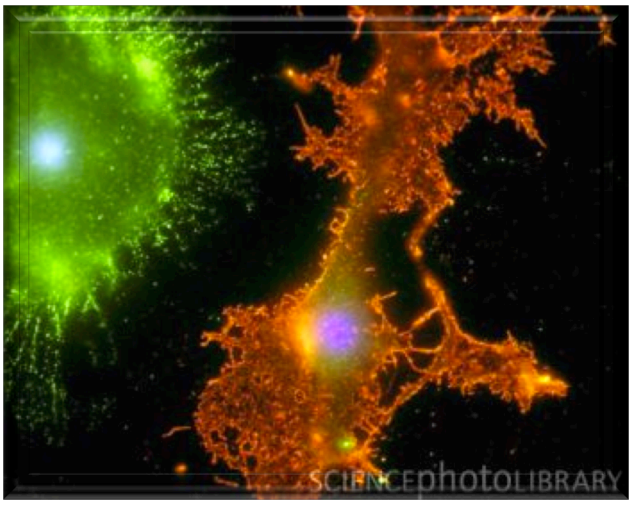

Figure: Fluorescent light micrograph of microglial cell (green) & oligodendrocyte (orange) (sciencephotlibrary)

Research indicates that astrocytes release neurotransmitters via the exocytosis of vesicles and through other non-vesicular pathways. For example, glutamate is released from astrocytes by the reversed activity of transporters under pathological conditions in which there is an increased intracellular concentration of sodium (or glutamate), coupled with

cell depolarization and an increased extracelluar concentration of potassium (Jourdain, et al., 2007).

Volume-activated anion channels also represent non-vesicular methods of releasing glutamate and other negatively charged amino acids. These channels open in response to hypo-osmotic shock, and remain highly important in the supraoptic nerve of the hypothalamus, which is the primary control center for body osmoregulation (Verkhratsky & Butt, 2007).

Vesicular release, the characteristic secretion of neurotransmitters by neurons, is calcium dependent. While neurons feature the rapid influx of calcium from the extracellular environment, glial cells appear to rely on the release of intracellular calcium stores for exocytosis (Verkhratsky & Butt, 2007). Thus, the release of glutamate via this pathway is markedly slower than in neurons due to its metabotropic nature. However, research indicates that glial cells posses all of the necessary proteins and structures required for exocytosis, including the SNARE and Snap proteins, as well as synaptic microvesicles and vesicular transporters (Herts, et al., 1999). Table 1.4 summarizes the most likely mechanism of release of several 'glio-transmitters'.

Table 1.4: Gliotransmitters & Their Release

Neurotransmitter	Mechanism of Release
Glutamate	Vesicular, Hemichannels
Aspartate	Hemichannels
ATP	Vesicular, Hemichannels
D-Serine	Unestablished
Homocysteine	Unestablished
Taurine	Volume-Activated Chloride Channels

(adapted from Verkhrastsky & Butt, 2007)

The fact that neuroglia are capable of releasing neurotransmitters has founded an explosion of research in this area that has provoked a new perception of synaptic organization in which neurons and glia, namely astrocytes, exist as equally important moderators of information transmission (Volterra, et al., 2002). This is now known as the tripartite synapse, in which three components have been identified: the presynaptic terminal, the postsynaptic terminal and the surrounding astrocyte. Together these constituents allow for the conduction of the neuronal post-synaptic potential, followed by the calcium wave propagation of the astrocyte and ensuing release of glio-transmitters that not only affect the surrounding glia, but also the pre and post-synaptic membranes of the synapse itself. This is indicative of bi-directional communication between neurons and glia, and is highly supported by current research (Volterra, et al., 2002).

As previously mentioned, both astrocytes and NG-2 glia are capable of forming synaptic contacts with neurons, as found in the hippocampus and the cerebellum. Thus far, two types of synapses have been found: glutamatergic and GABAergic (Verkhratsky & Butt, 2007). From these connections, it has been demonstrated that the stimulation of neurons produce the Ca2+ wave within the astroglia, which is then able to determine the intensity of the initial signal, as

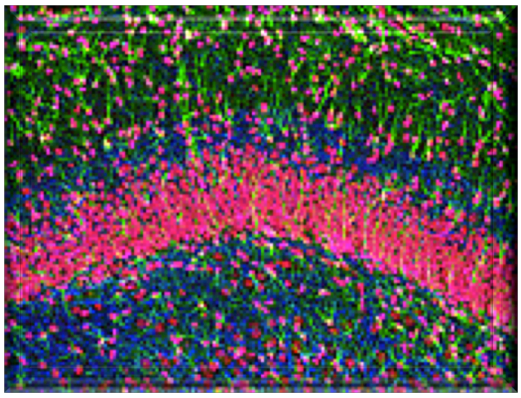

Figure: Light micrograph of sagittal hippocampal tissue; glial cells (green), neruofilaments (blue), nuclei (red) (sciencphotolibrary)

encoded by the Ca2+ wave oscillation. This phenomenon has been compared to the long-term potentiation seen in the hippocampus. However, instead of a change in neuronal amplitude, there is a deviation in glial calcium wave frequency coding (Verkhratsky & Butt, 2007).

The reverse communication direction, glia to neurons, implicates the ability of the propagating calcium wave to induce the release of glio-transmitters, which diffuse to affect neurons within the vicinity via ionotropic and metabotropic receptors (Volterra, et al., 2002). For example, the release of glutamate by astrocytes can directly depolarize a neuron via ionotropic AMPA and NMDA receptors. By acting on several neurons at once, this interaction may provide synchronous activation or inhibition of a group of neurons (Verkhratsky & Butt, 2007).

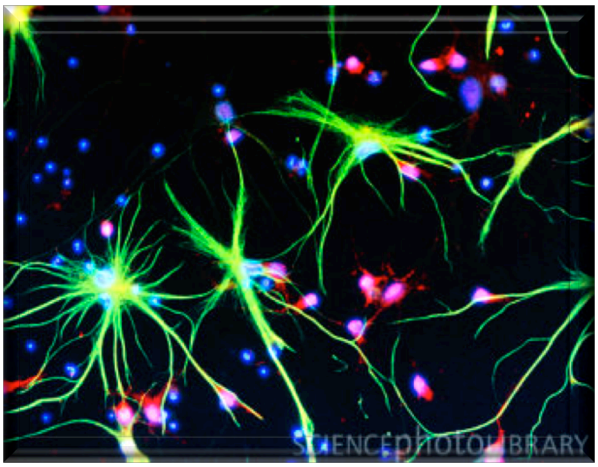

Figure: Immunofluorescent light micrograph of neurons & astrocytes (green) (sciencephotolibrary)

While it is clear that glia do modulate neuronal activity via release of these glio-transmitters, the phenomenon's importance in the overall integration of brain activity is still under intense scrutiny (Volterra, et al., 2002).

The communication between other types of glia, oligodendrocytes and Schwann cells in particular, has been far less studied. However, given that these cells rest directly on axons, the notion that there is no crosstalk is moot. In fact, it

is now known that the release of adenosine, ATP and glutamate from active axons influences the Ca2+ signals of oligodendrocyte progenitor cells to determine oligodendrocyte development and axonal myelination. Whether or not oligodendrocytes signal back to axons has not yet been established. However, it is hypothesized that they may release glutamate via hemichannels (Verkhratsky & Butt, 2007).

Schwann cells are highly integrated into the synaptic cleft, also relying on Ca2+ wave propagation as a means of excitation. For example, the high frequency stimulation of nerve terminals in the periphery triggers an increase in Ca2+ elevation, as mediated by neurotransmitter release (Verkhratsky & Butt, 2007). These signals then feedback to the neuron to either potentiate or depress synaptic activation via neuronal release of neurotransmitters. This mechanism has been implicated in the genesis of chronic pain (Malya, et al., 2007).

The over-arching characteristics highlighted above are applicable to most types of neuroglia. However, each sub-class also maintains several unique physiological hallmarks that allow the associated cells to accomplish targeted tasks, as well as contribute adverse consequences in neurological disease. The following section will focus largely on the innate physiological hallmarks of astrocytes, with minor discussion of oligodendrocyte and microglial physiology.

Chapter 4: Characteristic Physiology

Astrocytes are imperative to central nervous system function from the very beginning of development. Mulitpotent progenitors of astrocytes act as stem cells

not only during early development, but also during adult life, strictly in the hippocampus and subventricular zone of the human brain (Scemes & Spray, 2012). The primary difference between these progenitor astrocytes and mature astrocytes is the expression of the protein nestin (Verkhratsky & Butt, 2007).

Throughout development, the proliferation and migration of neuronal axons are dependent on astrocytes, including foetal radial glia. These astrocytes provide the scaffolding that neural precursors move along to find their final destination (De Keyser, et al., 2008). Also, channels laid down by astrocytes assist in this migration, providing a substrate for axon growth (Scemes & Spray, 2012). A highly important example of this is the glial sling, which allows neural precursors to cross hemispheres via the corpus callosum. Finally, axonal growth is further assisted by the release of membrane-bound and extracellular matrix molecules such as N-cadherins and lamnin-1 from astrocytes. These activate receptors on the axonal growth cones to promote the outgrowth of processes (Verkhratsky & Butt, 2007).

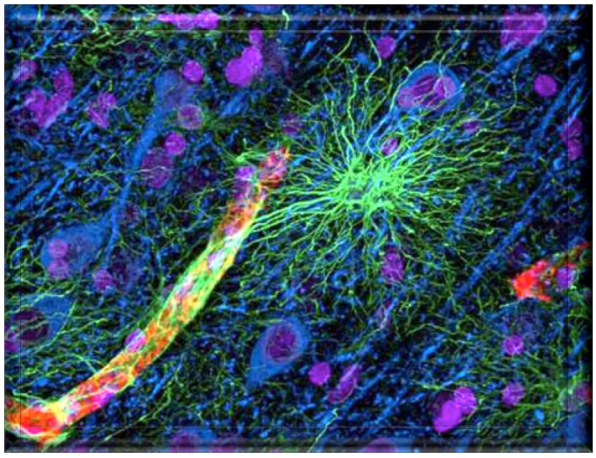

Figure: False color micrograph of astrocyte (green) making contact with a blood vessel (yellow/red), surrounded by neurons (blue). (msnbc)

Astroglial involvement in the migration of axons extends to influence synaptogenesis, which begins with the release of cholesterol (Verkhratsky & Butt, 2007). In vitro studies have found that the presence of astrocytes during

synaptogenesis increases the number of synapses formed by seven-fold. Astrocytes are also responsible for increasing the number of post-synaptic receptors and facilitating clustering via the release of TNF-α for glutamate and ADNF for NMDA receptors (Taupin, 2006). Overall, this modulates post-synaptic responses. Finally, synaptic elimination is a specific duty of astrocytes. This is accomplished when astrocytes completely cover a synaptic cleft and release abundant proteolytic enzymes, which consume the extracellular matrix, dismantling the synapse (Verkhratsky & Butt, 2007).

As previously stated, astrocytes in a healthy central nervous system maintain their own territory within both the brain and spinal cord (Wange & Bordey, 2008). If there is overlap between astroglia, it is always less than five percent. Thus, each astrocyte resides within its own microdomain, in which neurons, blood vessels and synapses are subject to segregation and influence by astrocytic processes, both lamellopodia and filopodia (Verkhratsky & Butt, 2007). These processes are motile and capable of covering anywhere between 100,000 to two million synapses. It is the extensive reach of these processes, coupled with

Figure: SEM of astrocyte (red) & neurons (blue) (sciencephotolibrary)

their molecular capabilities, which lend astrocytes their unique functions (Kettenmann & Ransom, 2005).

It has been established that astrocytes maintain a "metabolically independent glial-neurone-vascular unit (147)." This is due to one of the more well known functions of astrocytes in the formation of the blood-brain barrier. Located between the intracerebral blood vessels and the brain parenchyma, the blood-brain barrier is constituted by endothelial cells connected by tight junctions and astroglial endfeet that surround the capillary wall. The presence of astrocytic endfeet is by no means passive, as the release of regulatory molecules such as glial-derived neurotrophic factor, which actually modify the vascular wall, inducing the formation of tight junctions. This communication is bi-directional as the endothelial cells stimulate astrocyte maturation through the release of leukemia-inhibitory factor (Verkhratsky & Butt, 2007).

In addition to the interactions given above, astrocytes also maintain control over the tone of the vasculature itself. Astrocytes are responsible for functional hyperaemia. Calcium signaling, as induced by neuronal activity, travels to the astroglial endfeet where it initiates the release of vasoactive substances, namely arachindonic acid, which either causes vasodilation or vasoconstriction (Verkhratsky & Butt, 2007). Overall, the continuous contact maintained by astrocyte endfeet with the vasculature regulates blood flow, as well as the expression of not only ion channels within the glial membrane, but also the presence of specific receptors (Sofroniew & Vinters, 2010).

The fact that astrocytes express the same variety of ion channels and receptors as neurons do, allows astrocytes to regulate ion homeostasis for not only themselves, but also for the surrounding extracellular environment and thereby, neurons (Kettenmann & Ransom, 2005). Most importantly, astrocytes prevent the accumulation of potassium in the extracellular space. This is accomplished by two different mechanisms. First, there is a local uptake of potassium via potassium channels and transporters, including the sodium/potassium pump and the sodium/potassium/chloride pump. This type of transport, however, only accounts for a small portion of the entire reuptake of potassium. The majority of the potassium ion modulation occurs through spatial buffering. Astrocytes take potassium into the cell, where the ions are then redistributed to the interstitium or perivascular space via the gap junctions of the glial syncytium. Water is redistributed in much the same manner (Verkhratsky & Butt, 2007).

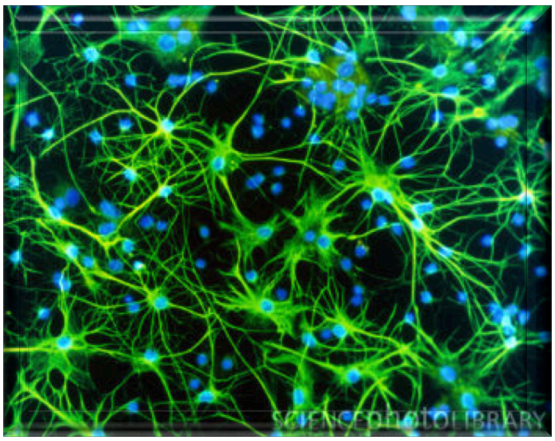

Figure: Immunofluorescent micrograph of astrocytes: cytoplasm (green) & nuclei (blue). (schiencephotolibrary)

Astrocytes also monitor the extracellular level of chloride, calcium and hydrogen ions through the activation of anion channels, transporters and aquaporins (Verkhratsky & Butt, 2007). Of particular importance is the regulation of extracellular glutamate. Due to the fact that high levels of glutamate are extremely toxic to the brain, the release and reuptake of glutamate is tightly

monitored (Herts, et al., 1999). Glutamatergic synapses are completely ensheathed by perisynaptic processes of astrocytes to prevent leakage of glutamate to neighboring synapses. Also, astrocytic membranes contain glutamate transporters, which account for eighty percent of the total amount of glutamate reuptake. Once the glutamate has been taken into the cell, it is converted to glutamine by the enzyme glutamine synthetase, and then released back to neighboring neurons via the glutamate-glutamine shuttle (Verkhratsky & Butt, 2007).

The ability of astrocytes to recycle molecules and shuttle them to neurons is not restricted to glutamate. In fact, research has demonstrated that astrocytes are critically involved in neuronal metabolic support. Astroglia account for only ten percent of the brain's energy consumption. However, there remains an equal distribution of glucose across neurons and glia, indicating an important role for astrocytes in energy metabolism of the brain (Kettenmann & Ransom, 2005).

This intermediate is the astrocyte-neuron lactate shuttle. Astrocytes process glucose via aerobic glycolysis into lactate, which is then transported to neurons via the monocarboxylase transporters. The neurons then use the lactate to produces seventeen ATP via the Kreb's cycle (Verkhratsky & Butt, 207). This mechanism is directly controlled by the extracellular level of glutamate released during synaptic transmission. Elevated levels of glutamate signal astrocytes to increase the delivery of energy metabolites to the active neurons (Herts, et al., 1999). It also directly stimulates the GLUT-1 transporters of the endothelial cells to double or even triple their uptake of glucose. Finally, astrocytes also contain the only store of

glucagon in the brain. During times of intense stimulation or stress, astrocytes are able to convert glucagon to glucose and shuttle it to neurons (Verkhratsky & Butt, 2007).

Since astrocytes have inched their way back into the limelight, theories have arisen that suggest that astrocytes may be a suitable substrate for memory and even consciousness (Verkhratsky & Butt, 2007). Some neuroscientists suggest that the binary code of neurons may not be suitable for the complex array of brain functions, especially consciousness and memory (Kettenmann & Ransom, 2005). It is argued that astrocytes, given their extensive connections via the glial syncytium, may provide a more diverse platform for information exchange, interpretation and modulation, as compared to neurons, which appear to be specialized for the transduction of information (Verkhratsky & Butt, 2007). With the current blossoming of research in the field of glial science, hopefully this astrocentric theory will be further pursued.

Moving on from astrocytes, the diversity of glial cell function diminishes greatly. The primary function of both oligodendrocytes and Schwann cells is the myelination of neuronal axons in the central and peripheral nervous systems, respectively. This is an interdependent phenomenon as signals to and from both glia and axons are responsible for the proper myelination of the nervous system. Oligodendrocytes are exclusively committed to myelination, while Schwann cells act similar to astrocytes as they send intermodal projections to the nodes of Ranvier to

modulate neuronal metabolism, signaling and the microenvironment (Armati & Mathey, 2010).

Myelin rests on axons as concentric layers of lamellae, extensions of the glial cell membrane. Seventy percent of myelin is composed of lipids, while the other thirty percent is comprised of proteins. The lipids lend myelin its unique insulating properties that make salutatory conduction possible. The proteins of myelin, namely myelin basic protein and proteolipid protein, allow the lamellae to stabilize and fuse (Verkhratsky & Butt, 2007). The degradation of myelin is associated with a number of disorders. The most well known being multiple sclerosis (Armati & Mathey, 2010).

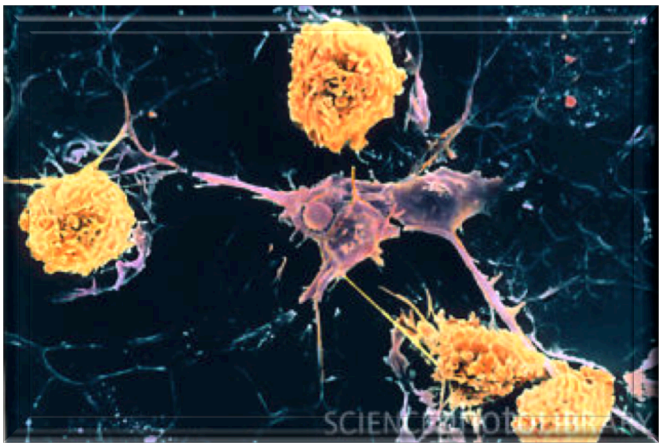

Figure: SEM of microglia (yellow) attacking an oligodendrocyte (purple) (sciencephotolibrary)

Each type of neuroglia utilizes their unique abilities to maintain central and peripheral nervous system function. However, recent research indicates that neuroglia, astroyctes in particular, play tremendously important roles in nervous system dysfuntion, from neurodegenerative diseases to psychiatric illness (Hamby & Sofroniew, 2010). The following section will examine the role that astroglia play in key nervous system pathologies including astrogliosis, Alexander's disease, and Alzheimer's disease.

Chapter 5: Pathology

Astrogliosis

A ubiquitous hallmark for central nervous system pathology is reactive astrogliosis (Verkhratsky & Butt, 2007). The precise definition of this marker is highly varied depending on the assault's intensity and severity. However, the general consensus is that astrogliosis is not an all or none response. Instead, the progression of reactive astrogliosis exists on a graded continuum, which features changes at both the cellular and the gene level. Broadly, reactive astrogliosis has been divided into three categories: mild to moderate, severe diffuse and severe reactive with compact glial scar (Sofroniew & Vinters, 2010).

For the past 100 years, the function of astrogliosis was largely viewed as a maladaptive mechanism because of its inherent ability to inhibit axonal regeneration following an insult. In fact, it was suggested that a therapeutic technique should inhibit the astrogliosis and scar formation itself. There are indeed further detrimental effects incurred by astrogliosis. Reactive astrocytes are capable of producing neurotoxic levels of reactive oxygen species, which are highly damaging to the neuronal environment. Also, cytokine production by reactive astrocytes exacerbates inflammation, while the release of

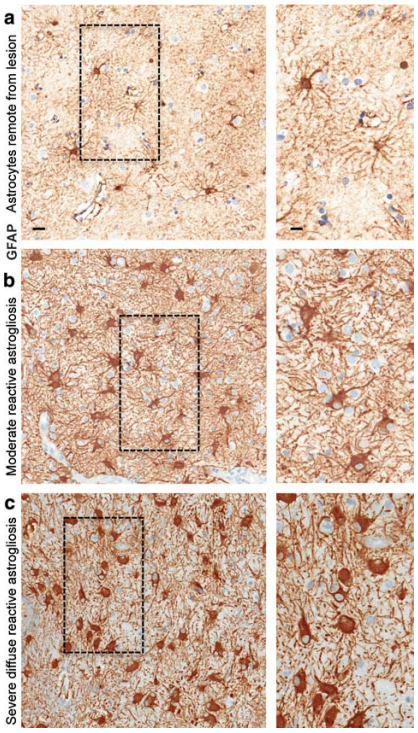

Figure: Progression of reactive astrogliosis (Sofroniew & Vinters, 2010)

excitotoxic levels of glutamate by reactive astrocytes is involved in the genesis of seizures (Zhang, et al., 2010).

Despite the challenges posed by these detrimental occurrences, current research indicates that the functions of reactive astrocytes and the glial scar actually protect the central nervous system through a variety of means, including the re-uptake of excitotoxic glutamate, repair of the blood-brain-barrier, stabilization of extracellular ion balance and the restriction of inflammation and spread of pathological agents. Reactive astrogliosis is seen in nearly any central nervous system disturbance, and has been shown to be of particular importance for strokes and tumors (Sofroniew & Vinters, 2010).

Alexander's Disease

Alexander's disease is the only example of a primary astrocyte disease, meaning that there is an inherent malfunction in the astrocytes. Resulting from heterozygous *de novo* mutations in the gene coding for glial fibrillary acidic protein, Alexander's disease is quite rare, affecting mostly infants. It is also usually fatal (Sofroniew & Vinters, 2010).

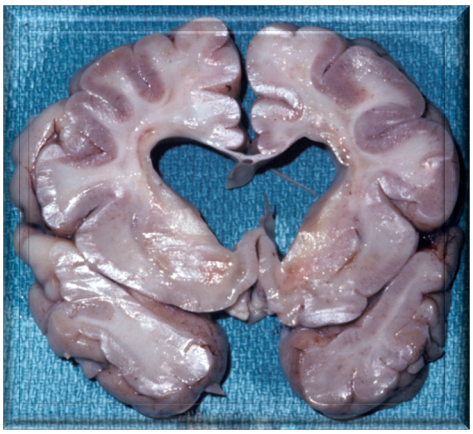

Figure: Infantile brain with Alexander's disease. (neuropathology-web.org)

Although the disease can affect people of all ages, who then present with different symptoms, the presence of Rosenthal fibers is a ubiquitous hallmark of the disease. Rosenthal fibers are cytoplasmic inclusions formed by GFAP, coupled with alpha and beta crystalline stress proteins, and heat shock proteins. Other common symptoms include hydrocephalus, convulsions, mental retardation and muscle weakness. After the on-set of the disease, life expectancy is between two to four years (Verkhratsky & Butt, 2007).

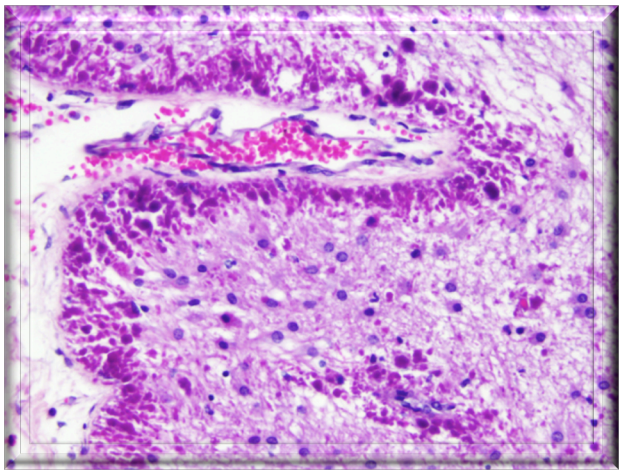

Figure: Rosenthal fibers in the subpial layer of the cortex. (neuropathology-web.org)

The mechanism of destruction and eventual death in Alexander's disease remains a mystery. However, it is often associated with rapid demyelination and a permanent disruption of the blood-brain barrier. In general, the missense mutation in the GFAP gene causes malformation of the cytoarchitecture of fibrous astrocytes, which then cannot perform their intended duties, eventually leading to not only astrocytic death, but also neuronal death (Ranson, et al., 2003).

Alzheimer's Disease

The potential involvement of glia in the pathogenesis of Alzheimer's disease was first suggested by Alois Alzheimer himself in 1910. However, like the rest of glial science, this was not fully investigated until the last twenty years

(Verkhratsky & Butt, 2007). The primary physiological characteristics of Alzheimer's disease are the accumulation of amyloid-beta plaques throughout the gray matter, as well as the formation of abnormal tau protein filaments, known as neurofibrillary tangles, in neurons (Fitzgerald, et al., 2012).

Research suggests that the accumulation of amyloid-beta plaques activate astrocytes, causing them to change from a basal state to a reactive form. This results in the exacerbation of an inflammatory cascade due to the fact that astrocytes may actually be innate immunocompetent cells of the central nervous system (Sofroniew & Vinters, 2010). Capable of expressing pattern recognition Toll-like receptors, astrocytes contribute to neuroinflammation via the release of cytokines, chemokines and nitric oxide. This is in conjunction with the activation of the recognized immune cells of the central nervous system, microglia, and their systematic release of cytotoxic mediators (Fuller, et al., 2009).

The study of astrocytic involvement in the progression of Alzheimer's disease has demonstrated that the release of pro-inflammatory molecules is only half of the story. As astrocytes morph from normal resting states to reactive immune-like cells, there is a decrease in their metabolic support of neurons, including the imperative recycling of excitotoxic glutamate. This leaves the neurons vulnerable to neurotoxins including elevated levels of cytokines and reactive oxygen species (Sofroniew & Vinters, 2010). In fact, the production of reactive oxygen species is increased with the activation of astroglia and their subsequent release of nitric oxide, as well as the characteristic microglia 'oxidative burst.' An elevated level of

reactive oxygen species is implicated in the progression of Alzheimer's disease, as ROS are responsible for DNA mutations and cytoarchitectural damage (Fuller, et al., 2009).

These findings suggest that astrocytes may be potential targets for therapeutic treatments. Most hypotheses focus on drug inhibition of astrocytic activation. Anti-inflammatory and anti-oxidative therapy drugs hold the greatest potential (Fuller, et al., 2009).

A second theory focuses on the interactions that astrocytes maintain with accumulating amyloid-beta plaques. When activated, reactive astrocytes follow the general pattern of astrogliosis to surround the neuritic plaques. Astrocytes also cover the plaque and interweave their processes into the core. Thus, the amyloid-beta plaques seen in Alzheimer's are not only composed of amyloid-beta proteins and degenerating neurites, but also astrogial processes and activated microglia (Sofroniew & Vinters, 2010). Recent research has revealed that astroglia not only sense and react to the deposits of amyloid-beta protein, but also use their overarching and interwoven processes to uptake the deposit and degrade the protein (Verkhratsky & Butt, 2007).

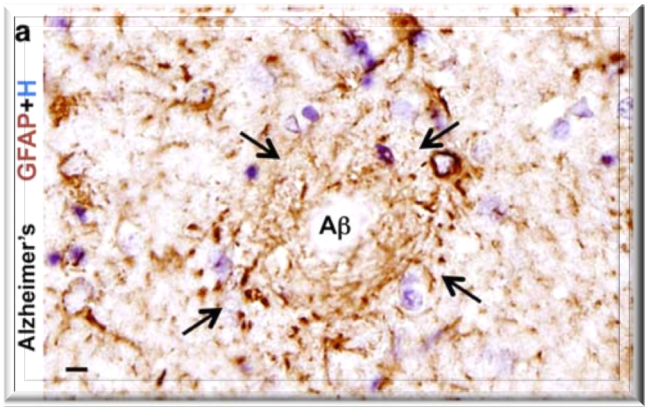

Figure: Reactive astrogliosis surrounding beta-amyloid plaque. (Sofroniew & Vinters, 2010)

One of the most recent theories of Alzheimer's disease progression posits that the early stages of AD feature an over-production of amyloid-beta protein by neurons, which then activates astroglia. Astrocytes work to clear the accumulated plaques and neuronal debris within their domains (Sofroniew & Vinters, 2010). However, there is a threshold whereby an astrocyte becomes overloaded and ceases to function, leading to a decreased level of care for neighboring neurons. As these neglected neurons shrivel under cytotoxic conditions, they too begin to release an increased amount of amyloid-beta protein. Once an astrocyte's domain has completely degenerated, the entire area undergoes lysis, facilitating further spread of the plaques (Verkhratsky & Butt, 2007).

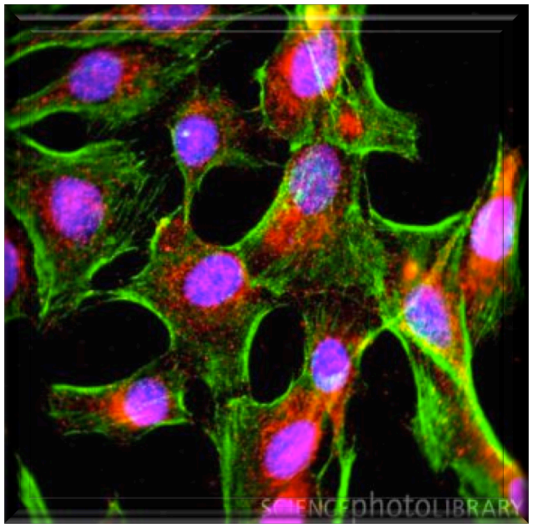

Figure: Fluorescent deconvolution micrograph of cultured glial cells expressing tau protein (red). (sciencephotolibrary)

As in many studied pathologies, it appears that astrocyte involvement in the progression of Alzheimer's disease is double-sided (Fuller, et al., 2009). The astrocytic supplementation of the inflammatory cascade via the release of cytokines further damages neurons, as does the reduction in metabolic support activity (Verkhratsky & Butt, 2007). However, the fact that astrocytes maintain the ability to not only remove the amyloid-beta plaques, but also degrade them, implies that there is an imperative neuroprotective mechanism (Sofroniew & Vinters, 2010).

Either theory provides sound potential for an innovative treatment of Alzheimer's disease through the manipulation of astrocytic physiology (Fuller, et al., 2009).

The involvement of glia, from astrocytes to microglia, in the vast array of pathologies studied today is now recognized as an incredibly intricate and complex notion. From Schizophrenia to Parkinson's disease, findings in basic glial research indicate incredibly diverse roles for glia in both resisting degradation and contributing to degeneration (Fields, 2009).

The scope of glial science is wide indeed. With tremendous diversity in histology and function, glial cells represent an untapped reservoir of potential for scientific research and clinical implication. Our technology is finally pulling the neuroscientific community out of the shadow of the Neuron Doctrine and its unchallenged dogma. While the integration of glia into the facilitation of such phenomena as consciousness and behavioral manifestations remains an opaque mystery, the question of glial passivity has been thoroughly debunked.

Although slow, the glial advocacy movement is gaining momentum. In the near future, perhaps we can hope for equal representation of the brain's 'other half' beside the cherished neuron. With the recognition that neuroglia are equally important contributors to nervous system function and pathology, perhaps glial science will become an established field of study, and earn neuroglia a much deserved extension on their typical paragraph depiction in premier neuroscience textbooks.

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