THE ALTERED ROLES OF GLIA IN NEURODEVELOPMENTAL DISORDERS

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Abstract:

For many decades in neuroscience, glial cells were thought almost exclusively to provide passive, primarily metabolic, support for the billions of neurons in our central nervous system (CNS). Today, however, the three types of CNS glial cells, astrocytes, oligodendrocytes, and microglia, are known to play a myriad of active roles that are necessary for normal neurodevelopment. Astrocytes have been found to regulate neurogenesis, support neuronal migration, promote axon elongation, guide the growth of processes, induce synapse formation, and modulate synaptic strength. Oligodendrocytes were thought to be important only for myelination of axons, but it has become clear that they are dynamic modulators of axonal conduction velocity and providers of metabolic support. Similarly, microglia were known almost exclusively as the immune cells of the CNS; however, recent research has exposed their importance in the regulation of cell number, cell differentiation, maturation of neural circuits, and synaptic remodeling. Recent findings suggest that aberrant glial cell function contributes to some of the unusual pathology seen in Noonan syndrome, schizophrenia, cerebral palsy, autism spectrum disorder, and other neurodevelopmental disorders. Moreover, new insights about the details of neuron-glia interactions allow for speculations about how altered glial cell function could actively contribute to abnormal neurodevelopment.

Introduction:

In order to understand the CNS deficits of patients with a neurodevelopmental disorder, one must first understand how the normally developing CNS forms. A cohesive understanding of this formation would involve the orchestration not only of neurons, but also of all the other cells that make up the CNS. It is becoming increasingly more evident that the other cells composing the CNS, specifically glial cells, are actively involved in the development, plasticity, and maintenance of the CNS. These cells should no longer be ignored in research that attempts to reveal what has gone awry in CNS development, for incorporating them could lead to more effective, comprehensive interventions for neurodevelopmental disorders.

The broad variation in disorders caused by abnormal development is an indicator of the huge complexity of the cellular interactions that underlie development. Similarly, the variation between individuals with the same neurodevelopmental disorders is likely due to subtle differences in these interactions. The intricate involvement of glial cells in developmental processes adds a level of complexity to this system and helps us to appreciate that cognitive dysfunction could be influenced not just by neuronal, but also by glial malfunctions. This review provides a critical summary of what is currently known about the roles of astrocytes, oligodendrocytes and microglia in normal neuronal development. The astrocyte population, for instance, is made up of functionally and morphologically diverse cells that are essential at multiple time-points throughout development and the life of the CNS. The myriad of roles that we now know astrocytes play completely rids them of the naïve reputation as passive cells. Oligodendrocytes were also thought to not do much beyond providing static myelin sheaths for axons; however, these cells have been revealed to continually adjust their myelin sheaths in response to neuronal activity, and they play some surprising novel roles. Similarly, all of the

nuances of microglia function that continue to be unraveled make it clear that they, too, influence most of the processes that are characteristic of CNS development. To exemplify exactly how the various types of glial cells could give rise to a specific pathology or behavioral phenotype, a handful of neurodevelopmental disorders are explored from the aspect of glial cell dysfunction.

A few specific glial cell functions are now known to be compromised in certain disorders; specific cases are explored in this review to support the notion that these cells may contribute to abnormal neurodevelopment. Additionally, a broader look at the involvement of each of these cell types in the normally developing CNS has allowed specific interactions to be identified as potential explanations for the physical and cognitive alterations that are manifested in individuals with specific neurodevelopmental disorders. Though specific roles for many of these interactions remain to be explored in detail, they are all good candidates given the current state of research. Overall, this review is intended to encourage acceptance of the strong possibility that glial cells are contributing to the altered neural development that leads to many devastating neurological disorders, and to emphasize that future research into disease mechanisms must include investigation of glia.

Astrocytes in Development:

The largest and most abundant type of glial cell in the CNS is the astrocyte. Astrocytes fall under two general categories. Protoplasmic astrocytes are found within grey matter and have many fine processes that create an overall globular structure. Fibrous astrocytes are distributed throughout all white matter, they also have fine processes but they are longer and much fewer in number than those of protoplasmic astrocytes (Ramon y Cajal, 1909). All astrocytes used to be thought of as merely the scaffolding that supports neural networks and the components of the

blood brain barrier (Wolburg et al, 2009). However, many more roles have been identified and it is now known that astrocytes have roles in a myriad of processes crucial for the development, health, and proper functioning of the nervous system.

Astrocytes are generated at a very early stage and have various roles that are important for the proper development of neural structures composing both the white and grey matter of the CNS. Several studies have shown that astrocytes function to regulate neurogenesis, support neuronal migration, promote axon elongation, guide the migration of growing processes and induce synapse formation. These findings will be considered below to provide a comprehensive picture of how astrocytes are involved in every step in formation of the nervous system.

Early in embryonic development, the generation of neuroblasts is followed by generation of astrocytes (Levitt and Rakic, 1980). Astrocytes then signal neuroblasts to differentiate and adopt appropriate neuronal fates (Lie et al., 2005; Song et al., 2002). Soon after they are born, neuroblasts must migrate away from the ventricular zone to the proper (Rakic, 1971). It is now understood that interactions between post-mitotic neurons and radial glial cells, a type of astrocyte, are necessary that migration (Rakic and Sidman, 1973; Nowakowski and Rakic, 1979). Once the young neurons arrive at their final destination, they must send out neurites (axons and dendrites) to make connections. Astrocytes are essential in this process, guiding growing processes by differentially expressing a variety of extracellular matrix molecules that either promote or inhibit elongation (Liesi and Silver, 1988; Snow et al., 1990; Steindler, 1993; Powell et al., 1997). This concert process is vital for the proper formation of pathways that make up biological neural networks.

Synaptic connections are another crucial element of functional networks that are formed and maintained with the help of astrocytes. Several adhesive proteins are known to play vital

roles in synapse formation and a few of these are expressed on the surface of astrocytes (Biederer et al., 2002; Sandau et al., 2011). The critical role of astrocytes in the formation of synapses has also been demonstrated *in vitro.* Retinal ganglion cells in culture formed up to seven times the number of synaptic connections when cultured on a layer of astrocytes compared to a culture without any glial cells (Ullian et al., 2001). The number of synapses must be finely tuned in order for neural networks to perform ideally. For instance, in animal models of both Fragile X syndrome (FXS) and Rett syndrome (RTT), an alteration in the number of functional synapses has been reported (Chao et al., 2007; Pfeiffer and Huber, 2007). These neurodevelopmental disorders are characterized by cognitive abnormalities exemplifying why tight control of synapse number is absolutely necessary.

Astrocytes continue to control and maintain the number of synaptic connections even after synapse formation. They remain closely associated with synapses, where they carry out neurotrophic roles including providing neurons with nutritional support and maintaining extracellular homeostasis (Araque et al., 1999; He and Sun, 2007; Parpura and Haydon, 2009). G.A. Banker (1980) demonstrated that the survival of neurons in culture was prolonged when astrocytes were present, indicating that astrocytes interact with the environment in a way that is beneficial to neuron longevity. One way they contribute to an environment ideal for neurons is by distributing necessary metabolites and molecules throughout the CNS. To accomplish this, blood must first travel through the area so the circulating metabolites and molecules can be distributed. Astrocytes control brain blood flow through their endfeet that line blood vessels. These endfeet induce vasodilation or vascular constriction in response to neuronal activity (Harder et al., 2002; Mulligan and MacVicar, 2004). Once the blood distributes metabolites and other molecules, how do they get into neurons and glial cells? There is not one clear answer to

this question, but astrocyte involvement has been supported by several findings.

Astrocytes express receptors and transporters that equip them to carry out activity-dependent uptake and distribution of certain molecules that are known to supply the brain with energy. The main molecule energetically utilized is glucose, and glucose transporters are dispersed on the surface of astrocyte endfeet (Murphy, 1993; Kacem et al. 1998). Various neurotransmitter transporters, including sodium-glutamate co-transporters, also are distributed over the surface of astrocytes (Bouvier et al., 1993; Morgello et al., 1995). Following the release of glutamate from excitatory synapses, glutamate is taken up by astrocytes along with sodium to stimulate glucose uptake and drive a coupled reaction that results in the breakdown of glucose into lactate (Pellerin and Magistretti, 1994). The transport of lactate from astrocytes to neurons is regulated by neuronal activity, and once it is transported into a neuron, it is utilized for energy-demanding processes that are necessary for cell function and viability (Pellerin et al., 1998).

An additional role astrocytes play in nervous system maintenance is in the sustenance of extracellular homeostasis. Astrocytes contribute to an environment that is ideal for neuron functionality in a few ways. One way is the removal of potassium ions from extracellular space (Kohuji and Newman, 2004). Glial cells have a resting membrane potential that is even more negative than that of neurons (Kuffler et al., 1966) and this property, coupled with the presence of inward rectifying potassium channels, allows astrocytes to take in potassium ions that accumulate outside active neurons and, if not cleared away, would slowly inactivate neurons (Verkhratsky and Steinhauser, 2000). Another feature that is important for this role, as well as others, is the astrocytic expression of connexins, which allows neighboring astrocytes to form gap junctions with one another (Giaume et al., 2010; Kuffler et al., 1966). This permits the formation of a network of interconnected astrocytes throughout which intracellular potassium

ions are redirected down their concentration gradient (Holthoff and Witte, 2000) and eventually back into the blood (Kohuji and Newman, 2004). When an ideal extracellular potassium ion concentration is not maintained, neurological disorders may result. Disorders, such as epilepsy (Scholl et al., 2009) and Alzheimer's Disease (Wilcock et al., 2009), are characterized by a decrease in the expression of astrocytic potassium channels. This abnormal phenotype supports the idea that astrocyte dysfunction may contribute to the cognitive abnormalities associated with these disorders.

Astrocytes are involved in homeostasis also through their uptake and recycling of neurotransmitters. Maintaining low extracellular levels of neurotransmitters is important for noise reduction in synaptic transmission and preventing over-excitation of neurons (Jabaudon et al., 1999). To do this, astrocytes express a variety of transporter proteins specific for important neurotransmitters and indeed do take up these neurotransmitters. Some of the neurotransmitters known to be taken up by astrocytes include GABA (Hosli and Hosli, 1979; Minelli et al., 1996), glutamate (Danbolt, 2001), histamine (Huszti et al. 1994), serotonin (Kimelberg and Katz, 1985), and norepinephrine (Kimelberg and Katz, 1986). However, all astrocytes throughout the CNS do not identically express transporters for these neurotransmitters. In fact, the level at which each neurotransmitter is taken up by astrocytes varies across brain regions (Kimelberg and Katz, 1986). This is most likely related to the variation in transmitters used in different regions of the CNS. Additionally, the uptake and release of some of these neurotransmitters by astrocytes is dependent upon the extracellular and intracellular environment of the astrocytes. For instance, the concentration of potassium and sodium ions affects whether histamine is taken up or released (Huszti et al., 1994). This is another example of modulation of the extracellular environment in

response to what astrocytes sense in their surroundings, which is an absolutely essential ability for a cell that is responsible for maintaining homeostasis.

Astrocytes also integrate neuronal signals to modulate synaptic function, which can be achieved through either presynaptic or postsynaptic modifications. Strengthening of presynaptic function can result from a change in the probability of neurotransmitter release and/or the size of the readily releasable pool. Astrocytes have been shown to participate in these modifications at certain types of synapses. For instance, cholesterol released from astrocytes has been seen to increase both the quantal content and the probability of neurotransmitter release in glutamatergic synapses (Mauch et al. 2001; Goritz et al., 2005).

A more recently identified presynaptic modification that astrocytes are required for is a phenomenon known as muting. This involves a reduction in the number of neurotransmitter-releasing presynaptic terminals and is induced by alterations in neuronal activity, such as changes in depolarization or prolonged activation of G-proteins (Crawford et al. 2011; Moulder et al., 2004; Moulder et al. 2006). Cultures of rat hippocampal neurons deprived of astrocytes did not exhibit muting in response to either depolarization or G-protein-coupled receptor activation. Muting was rescued with the induction of astrocyte-conditioned medium and also with the astrocyte-derived glycoprotein, thrombospondin (TSP) (Crawford et al. 2012). Molecules derived from astrocytes can alter the presynaptic terminal to result in either an increase or a decrease in synaptic efficacy.

The other side of the synapse can also be modified to create similar effects on synaptic strength. One effective way the postsynaptic terminal can be strengthened is by the addition and securing of receptors at the synapse. Proteins and other small molecules produced by astrocytes are known to contribute to these processes in response to synaptic activity. The expression and

stability of NMDA receptor subunits at glutamatergic synapses are controlled by activity-dependent neurotrophic factor (ADNF), which is synthesized in astrocytes in response to specific neurotransmitter activation (Blondel et al., 2000). Astrocytes also produce tumor necrosis factor α (TNFα), which increases synaptic efficacy by inducing AMPA receptor expression at the surface of the postsynaptic terminal (Beattie et al., 2002). More over, two proteins produced by astrocytes, glypican 4 and 6, were shown to be both necessary and sufficient to drastically increase the number of surface AMPA receptors containing the GluA1 subunit (Allen et al., 2012). So not only are astrocytes involved in postsynaptic remodeling processes, but they are also the key factors in specific changes that alone can lead to modulation of synaptic strength. They engage in these processes in response to specific signals and their response varies based on the nature of the signal. TSP and cholesterol, for example, do not have the same effect on inhibitory GABAergic synapses as they do on excitatory glutamatergic synapses (Hughes et al., 2010). Instead, brain-derived neurotrophic factor (BDNF) produced by astrocytes has been identified as a modulator of postsynaptic strength in inhibitory synapses (Elmariah et al., 2005). This further supports that astrocytes integrate signals and act to refine synaptic strength accordingly.

The way in which astrocytes respond to a signal has been of particular interest, but until recently exploring this topic was limited by experimental feasibility. The innovation of calcium imaging techniques revealed that astrocytes respond to synaptic neurotransmitters and other stimuli by increasing intracellular Ca^{2+} concentration (Cornell-Bell et al., 1990; Dani et al,, 1992; Pasti et al., 1997). This intracellular signal is not locally confined because Ca^{2+} waves can traverse astrocyte networks through gap junctions (Finkbeiner, 1992). This finding ultimately allowed us to accept astrocytes as actively communicating cells and gave rise to another type of

communication in the CNS known as gliotransmission (Bezzi et al., 2001). In response to Ca^{2+} signaling, astrocytes release various molecules into the extracellular environment. These kinds of molecules, which are known as gliotransmitters, include ATP (Zhang et al., 2003), glutamate (Bezzi et al., 1998; Jourdain et al., 2007) and d-serine (Yang et al., 2003). Each gliotransmitter has different modulatory effects on other glia, neurons and vascular cells. At synapses, their modulatory actions involve either the stimulation or inhibition of synaptic transmission that underlies plasticity. The secretion of these molecules from astrocytes has been shown to contribute to both long-term potentiation (LTP) and long-term depression (LTD), two phenomena that are absolutely essential for learning and memory (Han et al., 2012; Henneberger et al., 2010; Min and Nevian, 2012; Navarette et al., 2012; Panatier et al., 2006).

The ongoing study of astrocytes continues to reveal that this class of glial cells has many diverse functions that are necessary at multiple time points throughout the life of the nervous system. This includes developmental periods in which astrocytes regulate neurogenesis, support neural migration, promote axon elongation, guide growing processes, and induce synapse formation. Their involvement continues on past maturation of the CNS where they maintain extracellular homeostasis and modulate synaptic activity that is crucial for the proper functioning of neuronal structures.

Oligodendrocytes in Development:

Nervous system function relies heavily on the rapid transmission of signals. The speed at which this occurs in a specific axon is known as the axonal conduction velocity. This characteristic is dependent upon on a few variables, but is most notably determined by the degree of myelination. Myelin provides insulation to speed up the propagation of action potentials down

an axon. It does this by increasing the resistance of the membrane and, therefore, increasing the length constant. Capacitance is also influenced because myelin increases the distance between ions propagating down an axon and charges outside the axon, which allows ions to flow with less interference from external charges. However, at the nodes of Ranvier, the propagating ions are separated from external charges by the thin axonal membrane. At this point, the ions slow because of their electrical interactions with external charges. Once the node of Ranvier becomes fully saturated with charges, the ions are able to continue on and jump through the next segment of myelinated axon. This phenomenon is known as saltatory conduction. The thickness of the myelin sheaths and internodal length differences are two ways that myelin can differ between axons and result in varying conduction velocities (Smith and Koles, 1970).

Oligodendrocytes have long been known to form the myelin that surround neuronal processes (Penfield 1924), but the traditional view of myelin as a static insulator had to be re-evaluated after it was shown change in response to neuronal activity (Fields, 2013). Instead, oligodendrocytes had to be thought of as dynamic cells that both influence and are influenced by activity. They are capable of integrating information about the activity of neighboring neurons to remodel myelin appropriately (Demerens et al., 1996; Stevens et al., 1998). Additionally, these glial cells have been shown to provide metabolic support that is essential to the longevity of both individual axons and neurons (Morrison et al., 2013). These roles are necessary for the ideal performance and plasticity that allows for proper cognitive functioning.

Before understanding how oligodendrocytes provide metabolic support and regulate the degree to which an axon is myelinated, it is pertinent to know the features of oligodendrocytes and how they form myelin. Oligodendrocytes are multipolar glial cells that arise from oligodendrocyte precursor cells (OPCs) that have migrated into developing white matter from the

neuroepithelium (Barres and Raff, 1999). The proliferation of OPCs is dependent upon neuronal activity. This was proven by the finding that eliminating axonal electrical activity by either removing neurons or by pharmacologically blocking their activity leads to a drastic decrease in the number of OPCs (Barres and Raff, 1993). This reveals that neuronal activity dictates the location and amount of myelin early in development. During migration, OPCs are repelled by each other to result in an even distribution of these cells (Hughes et al., 2013). After settling into a defined territory, the OPCs can differentiate and form immature oligodendrocytes. However, the time at which the differentiation occurs depends upon the development of neuronal networks. Once neurons in a network become electrically active, OPC differentiation is stimulated by the removal of inhibitory transcription factors with microRNAs and other signaling pathways (Gibson et al., 2014; Piaton et al., 2010; Twak et al., 2011; Zhao et al., 2010).

From here, oligodendrocytes compete for growth and survival factors derived from both astrocytes and neurons (Barres et al., 1993; Barres and Raff, 1999). Astrocytes release signals required for the survival of immature oligodendrocytes in the early stages of development (Barres et al., 1994; Gard and Pfeiffer, 1993), but later on, oligodendrocyte survival is dependent primarily upon interactions with neuregulins on the surface of axons (Fernandez et al., 2000). Therefore, an oligodendrocyte must contact an axon to survive. This mechanism allows for the sustenance of the precise number of oligodendrocytes needed to fully myelinate a population of axons. The transition of an oligodendrocyte from an immature-unmyelinating cell to a mature-myelinating one is a relatively quick process. In fact, an oligodendrocyte can generate the entire myelinating process on one axon within five hours (Czpoka et al., 2013), which is impressive considering oligodendrocytes typically construct 20 to 60 myelin sheaths (Matthews and

Duncan, 1971). Once they have extended their processes, how do they decide which axons to myelinate?

This is a question that still lacks a comprehensive answer. However, the extensive knowledge about how Schwann cells, the myelinating cells in the peripheral nervous system (PNS), choose which axons to myelinate has led to a few insights about selective myelination in the CNS (Simons and Lyons, 2013). Two studies have collectively proven that axon size alone can initiate myelination by showing that oligodendrocytes readily myelinate axons with diameters greater than 400 nm even in the absence of any type of signaling (Almeida et al., 2011; Lee et al., 2012). Why this is the case is still unknown, but some speculate that is due to physical constraints of the oligodendrocyte cytoskeleton (Umemori et al., 1994). Although the necessary signals for myelination have not been identified, there are molecules that have been shown to either positively or negatively influence the myelination of axons that are both large or small in diameter (Michailov et al., 2004; Taveggia et al., 2005). The level of expression or function of some of these signals, such as Neuregulin 1 and Fyn kinase, are regulated by neuronal activity and this implies that activation indirectly influences myelination (Liu et al., 2011; Wake et al., 2011). Additionally, increased expression of Neuregulin 1 due to neuronal activity has also been seen to increase the thickness of myelin sheaths (Brinkmann et al., 2008). In sum, oligodendrocytes preferentially wrap their myelinating processes around axons with a large diameter, but are also influenced by activity-regulated signals to determine which of the smaller axons need to be insulated and to what degree.

After ensheathment, cell-cell interactions cause the axon and the oligodendrocyte to undergo structural changes or alterations in protein expression (Black et al., 1982; Laursen et al., 2011). These changes are thought to occur in preparation for necessary heterocellular

interactions. One important interaction is metabolite exchange. There are a variety of studies supporting that many metabolites originating in neurons translocate into oligodendrocytes and vice versa. The metabolite N-acetylaspartate (NAA) is an example of such a metabolite. It is synthesized in neuronal mitochondria, but is broken down in oligodendrocytes during the synthesis of myelin lipids (Moffett et al., 2007, Jalil et al., 2005). Metabolites also travel in the opposite direction: metabolites originating in the glial network are supplied to axons. For example, lactate from oligodendrocytes is translocated through a monocarboxylate transporter 1 (MCT1) into the periaxonal space for neuronal uptake through monocarboxylate transporter 2 (Lee et al., 2012; Pierre et al., 2000). The lactate that is supplied by oligodendrocytes is essential for axonal survival as proven by the degeneration of motor neurons in culture when MCT1 transport is inhibited. Additionally, patients with amyotrophic lateral sclerosis (ALS) have decreased expression of MCT1s in affected brain regions, which may reflect the importance of oligodendrocyte metabolite donation (Lee et al., 2012). An obvious follow up question would be: how do these metabolites get to oligodendrocytes?

The metabolites that oligodendrocytes provide are imported through the unidirectional gap junctions that connect them to the astrocytes (Ranson and Kettenmann, 1990; Robinson et al., 1993). This coupling of glial cell types comprises what is referred to as the panglial network and it creates a physical connection between oligodendrocytes and the nutrient-rich vascular system that astrocytes contact (Saab et al., 2013). Axon survival as well as myelination depends upon the connections formed by gap junctions. This is made evident by the finding that genetic deletion of oligodendrocyte connexins in both mice and humans results in severe hypomyelination as well as a decrease in axon longevity (Menichella et al., 2003; Uhlenberg et al., 2004). Pelizaeus–Merzbarcher-like disease (PMLD), a genetic cause of leukodystrophy,

results from a mutation in the gene encoding for an oligodendrocyte connexin (Uhlenberg et al., 2004). These outcomes strongly reinforce the importance of the metabolic exchange between neurons and the panglial network for the proper functioning of the nervous system.

Oligodendrocytes have yet another dynamic role that is much more complex and less understood. Currently, it is evident that neuronal activity drives the modification of myelin. Oligodendrocytes can alter myelination by adjusting sheath length, altering sheath thickness, wrapping unmyelinated axon segments or unwrapping myelinated ones. Changes in myelination have been seen using MRI techniques to visualize changes in white matter as a result of cognitive development or learning (Fields, 2008). Evidence of activity-dependent myelin remodeling has also been provided at the cellular level. For instance, neuronal activity has been shown to trigger changes that affect the size of the nodes of Ranvier by modifying the length of internodal myelin sheaths (Wurtz and Ellisman, 1986). This specific change would influence the degree of insulation and, therefore, affect the axon's conduction velocity. Myelin remodeling can also adjust the length and relative position of the axon initial segment (AIS, where the spike is initiated), which affects the axon's firing frequency and the shape of the action potential (Grubb and Burrone, 2010; Kole and Stuart, 2012; Yoshimura and Rasband, 2014). The signaling features of an axon can therefore be modified by activity-dependent myelin remodeling through cell-cell interactions. Presently, there is no direct evidence relating neuronal activity to changes in myelin sheath thickness or illuminating the mechanism that drives this form of remodeling. However, it is a likely form of myelin plasticity and has been suggested by studies that induced activity deprivation in animals and saw that these animals had thinner myelin sheaths in comparison to controls (Canu et al., 2009; Makinodan et al., 2012;). This implies that neuronal

activity has an effect on the thickness of the myelin sheath and as a result, affects the axon's conduction velocity.

Oligodendrocytes are able to monitor neuronal activity because they are equipped with membrane receptors, ion channels and neurotransmitter receptors (Barres et al., 1990; Karadottir et al., 2005; Ransom and Orkand, 1996). It is known that adenosine (Stevens et al., 2002), glutamate, and GABA (Berger et al., 1992) all influence oligodendrocytes in some way. Although it remains unknown where these neurotransmitters are released and how they get to oligodendrocytes, it has been hypothesized it is via non-synaptic transmission. The integration of these signals is likely what influences the specific changes in myelination previously discussed. This activity-dependent myelin remodeling is another form of plasticity that is vital for learning and memory. Synaptic plasticity, the more popular form, is a fundamental mechanism for learning and memory and it is well established that changes in synaptic strength depend upon the coincidence detection that results from the simultaneous arrival of two signals. For that to occur, the two action-potentials most likely must travel through neuronal networks at different speeds and myelin remodeling can readily adjust those velocities. This infers that myelin-determined conduction velocity is essential for synaptic plasticity to occur (Fields, 2015). In other words, oligodendrocytes' ability to alter an axon's conduction velocity in response to neuronal activity is necessary for the coincidence detection that generates changes in synaptic strength.

In all, oligodendrocytes are responsive cells whose roles are vital in the survival and proper functioning of the nervous systems. Moreover, the exchange of metabolites between neurons and oligodendrocytes has a prolific effect. When this exchange is disrupted, there is a drastic decrease in both axon and neuron longevity. Oligodendrocytes also integrate information regarding neuronal activity to selectively myelinate axons and to appropriately remodel myelin.

The importance of these functions have been previously discussed, but are additionally demonstrated in disorders that affect myelin in the CNS. A decrease in the number of oligodendrocytes due to genetic or environmental causes is known to result in nervous system dysfunction. This is shown in demyelinating diseases such as multiple sclerosis (MS) (Barnett et al., 2004). The aberration in nervous system function that is characteristic of neurodevelopmental disorders could also arise due to some deviation in oligodendrocyte function. A more nuanced understanding about oligodendrocyte involvement in the CNS could potentially shed light on this notion.

Microglia in Development:

Phagocytic cells in the CNS were first observed in the mid 1800's by Rudolf Virchow and later characterized in 1899 by Franz Nissl with the use of his new staining technique. He proposed that these rod-like cells play a role in migration, proliferation and phagocytosis (Nissl, 1899). Then early in the twentieth century, W. Ford Robertson coined the term "mesoglia" to describe cells that originated from the mesoderm instead of the ectoderm (Robertson, 1900). Shortly thereafter, these mesoderm-derived phagocytes were redefined as "the third element of nervous system" by Santiago Ramon y Cajal in 1913. These phagocytic elements finally were deemed "microglia" in order to distinguish them from oligodendrocytes, which also were thought to originate from the mesoderm (Del Rio-Hortega, 1919). This distinction was not easily accepted until Del Rio Hortega utilized silver staining techniques to characterize two different cell types that differed in both form and function. One cell type, referred to as oligodendroglia, lacked phagocytic features. The other cell type was microglia, which were seen to migrate and undergo phagocytic activity (Del Rio-Hortega, 1932).

The exact origin of these microglia remained a controversial topic for some time as several different hypotheses, each supported by findings, were advanced. A handful of studies supported the hypothesis that microglia and astrocytes share a common origin (Fujita and Kitamura, 1975; Hao et al., 1991; Fedoroff et al., 1997). Furthermore, one of these studies indicated that the common origin was actually the ectoderm (Kitamura et al., 1984). However, a preceding hypothesis suggested that microglia originate from blood vessel-associated pericytes (Baron and Gallego, 1972). Del Rio-Hortega's mesoderm-origination theory was also well supported (Murabe and Sano, 1982) and it was this body of research that illuminated the similarity between microglia and macrophages. Microglia were observed to be morphologically similar to macrophages (Murabe and Sano, 1983), to express macrophage markers (Perry et al., 1985; Akiyama and McGeer, 1990), and to have a genetic relationship to a subset of macrophagic cells (McKercher et al., 1996; Beers et al., 2006). These findings solidified microglia's similarity to macrophages; however, their precise origin remained a subject for debate. Since the beginning of the twenty-first century, the mesodermal, ectodermal and other origination theories have been disproved and bone marrow-derived monocytes were then widely accepted as microglia progenitors (Kaur et al., 2001). Beers et al. supported this origination theory in 2006 when they demonstrated that mutant mice that lacked embryonic microglia could generate microglia following a bone marrow transplant.

Though bone marrow-derived monocytes are still accepted to be progenitors of microglia, another origination theory has since been reconsidered. In 1999, Alliot et al. saw the presence of microglia progenitors very early in development in the yoke sac. This finding was seemingly overlooked until more recently, when fate-mapping experiments showed that haematopoietic stem cells in the yoke sac give rise to microglial progenitors early in development under

homeostatic conditions (Kierdorf et al., 2013). These studies also showed that bone marrow-derived monocytes contributed only slightly to the microglia population, which suggests that the majority of microglia progenitors originate from the yolk sac (Ginhoux et al., 2010). Other myeloid cells also originate from these haematopoietic stem cells and differentiate according to both endogenous and environmental factors (Geissmann et al., 2010; Sieweke and Allen, 2013). The various distinct populations of myeloid cells, microglia included, share many of the same myeloid and macrophage-specific markers (Prinz et al., 2011). Additionally, they all share a similar function: to detect and engulf pathogens, dying cells and other debris (Hickey et al., 1992). However, these populations have been shown to be genetically heterogeneous and to carry out different transcriptional regulatory pathways (Gautier et al., 2012).

Microglia differ from the myeloid cells that reside in tissues outside of the CNS in a few, distinct ways. First, the microglia progenitors that are detected early in embryonic development have been seen to divide rapidly and plateau late in gestation. Microglial cells arise midway through gestation from their progenitors and by just two weeks following birth, a large proportion of the microglia population has already been produced. During this developmental period, many of these differentiated microglia cells were determined to be mitotic (Alliot et al., 1999). This last finding led some to believe that microglia maintain the majority of their population by continuous self-proliferation instead of by differentiation from blood-derived monocyte precursor. However, there have been various contradictory findings in relation to this idea. Bone-marrow transplants have been used to explore the origin and regulation of microglia populations. A handful of studies have shown that a bone-marrow transplant results in the replacement of native microglia with donor-derived cells (Biffi et al., 2004). Other studies have shown the opposite, in which relatively few microglia are replenished by the transplant (Ajami et

al., 2007; Ginhoux et al., 2010). More recent studies have used genetic techniques to prove that the replenishment of the microglia population within the CNS occurs independently of blood marrow-derived monocytes. This finding solidified the notion that microglia are a self-perpetuating population.

Microglia that arise from haematopoietic stem cells in the yolk sac do not colonize the CNS until vascularization of this system (Marin-Padilla et al., 1985). In human fetuses, this takes place by 12 gestational weeks (Rezaie, 2003). During this time, intercellular communication aids in the migration of microglia toward developing vessels, where they promote the angiogenic process (Rymo et al., 2011). By the 12th gestational week, these immature, amoeboid-like microglia can be found within the subplate of the cerebral wall of the telencephalon, just below the meninges that surround the developing CNS. This time point in development also coincides with the formation of radial glia as well as the beginning of neuronal migration. Microglia move along the vasculature and, like immature neurons, they also have been shown to migrate alongside radial glia and white matter tracts during this phase in CNS development (Rezaie and Male, 1999). These migratory movements of amoeboid microglia have been shown to occur in two different migration steps: tangential then radial migration. These steps have been well documented in the retina of developing quail in which tangential migration of amoeboid microglia consisted of movement that was parallel to axons composing the retinal axon and cerebral white matter tracts (Navascués et al., 1995; Cuadros et al., 1997). Once microglia cease their migration in this direction, they proceed to migrate radially toward the pial surface (Navascués et al., 1996). This two-step migration has been observed only in areas of the brain composed of distinct layers, so it is possible that a different migration patterns take place in regions without laminar organization. There have been some implications that microglia

migration in mammals follows a similar pattern to that seen in quail (Milligan et al., 1991; Cavalcante et al., 1995), but no one has been able to directly observe this phenomenon in any other species.

These immature, amoeboid microglia eventually begin to give rise to a more mature type of ramified microglia that shares fewer similarities to other macrophages (Giulian, 1987). The amoeboid-like microglia can be found in the telencephalon even up to 24 gestational weeks, but are predominantly located in the ventricular zone as well as the subventricular zone. It is the ramified microglia population that resides in the subplate at later time points in fetal development (Rezaie et al., 2005). The colonization of the subplate zone by mature microglia corresponds temporally the to migration of neurons into this region and synaptic formation (Kostovic and Judas, 2002). It seems likely that signals are present during this migratory process to influence where microglia migrate. It was speculated that signals of this sort originate from cells undergoing apoptosis. This theory arose from findings that showed accumulations of microglia in regions of the parenchyma that had experienced a relatively large amount of cell death (Hume et al., 1983; Ashwell, 1990). However, the characterization of microglial migration in the developing quail retina (Navascués et al., 1996; Cuadros et al., 1997) as well as the lack of a relationship between microglia cell density and amount of cell death has invalidated this theory (Lawson et al., 1990; Marín-Teva et al., 1999).

It is likely that various signals influence the microglia migration, especially considering the various protein families present within the subplate zone, such as extracellular matrix proteins (including laminins), chemoattractants and chemorepellants. Neuron growth and survival is known to rely on many of these protein signals (Kostovic and Judas, 2002) and it appears that microglia also depend on some of them for successful differentiation, migration and

proliferation (Paolicelli et al., 2011; Hoshiko et al., 2012; Rezaie and Male, 1999). In vitro, it has been demonstrated that some chemokines (MCP-1, macrophage inflammatory protein and fractalkine) stimulate migration of microglia via cytoskeleton reorganization (Badie et al. 1999; Cross & Woodroofe 1999, Maciejewski-Lenoir et al. 1999). Although some of these factors, such as monocyte chemoattractant protein-1, are produced in response to injury of the immature CNS (Ivacko et al., 1997), it cannot be concluded that all microglial migration is a response to cell death, because there have been observations of tangential migration of microglia toward regions that lack dying cells (Marín-Teva et al., 1998).

More recent findings have further supported that it is not cell death alone that induces microglia migration. Using genetic techniques, Arnò et al., (2014) has shown that early neuron progenitors produce a chemokine that is necessary for the migration of immature microglia to the subventricular and ventricular zones during the development of the cerebral cortex. Sex-specific mechanisms may also influence microglial migration. There are reports of a difference in the cell number and distribution of microglia in the developing brains of male and female rats (Schwarz et al., 2012; Lenz et al., 2013). This finding identifies a possible a reason why one sex is more susceptible to the development of certain neurological disorders than the other. Although the sex-specific mechanisms or factors that influence the microglia population are unknown, these findings further support that microglia migration and differentiation is a very complex process. In sum, the orchestration of microglial migration during development remains incompletely described, but observations that factors released independently of apoptosis contribute to the localization of microglia suggest that these cells have other responsibilities in addition to the digestion of debris and dying cells.

A more nuanced understanding of the other roles microglia play in the CNS has come about in the last twenty years. As of today, it is known that inactive microglia are important in the regulation of cell number, differentiation of various cell types, maturation of neural circuits, and synaptic remodeling. The activation of microglia due to an immune response causes them to slightly alter their influence on these processes. Most interestingly, activated microglia influence pruning and axon outgrowth to a different degree. Taken together, all of these roles make it clear that microglia should not be ignored when considering possible contributors to the aberrant connectivity and functionality seen in the diseased CNS.

Microglia control the number of both neurons and other glial cells in the CNS in a few, seemingly contradictory, ways. First, they have control over normal programmed cell death of neurons. This function was revealed by a remarkable decrease in the amount of programmed cell death that occurred in a chick retina cultured without microglia. The addition of microglia into this culture resulted in an increase in retinal cell death, and this result was determined to be due to the presence of a microglia-derived nerve growth factor (Frade and Barde, 1998). In other regions of the CNS, different microglia-derived factors are responsible for the programmed cell death of other types of neurons (Marin-Teva et al., 2004; Sedel et al., 2004). Microglia have long been known to engulf dead and dying cells (Ferrer et al., 1990), but their active role the initiation of cell death is a relatively newer discovery. Moreover, *in vivo* studies have suggested that microglia primarily regulate programmed cell death in regions undergoing neurogenesis (Sierra et al., 2010) and then clear away excess neural progenitor cells once neurogenesis is nearly complete (Cunningham et al, 2013).

Other reports claim that microglia promote cell proliferation and maturation through the production of trophic and anti-inflammatory factors (Liao et al., 2005; Morgan et al., 2004;

Polazzi et al., 2001). A significant decrease in neural progenitor cell proliferation was seen in a cultures lacking microglia, but proliferation was increased with the addition of microglia to these same cultures (Antony et al., 2011). Together, the reports of pro-apoptotic as well as trophic effects of microglia suggest that these glial cells tightly regulate the number of neurons in many regions of the CNS. It is speculated that microglia may either have different effects on neurons in different brain regions or that the population of microglia is heterogeneous and therefore, their influence varies from region to region (Frost and Schafer, 2016). Unearthing an explanation of the opposing roles of microglia would aid in the understanding of the role of microglia in the developing as well as diseased brain.

Microglia also influence the development of other glial cells by influencing the differentiation of progenitors into either astrocytes or oligodendrocytes. It has been shown that more astrogenesis occurs in populations of neural progenitor cells when cultured in microglia-conditioned media, due to the presence of microglial-derived factors such as interleukin (IL)-6 (Nakanishi et al., 2007). Oligodendrocyte precursor cells (OPC) also have increased differentiation into mature oligodendrocytes when cultured in microglial-conditioned media. Interleukin (IL)-6 has also been identified to contribute to this event along with several other microglial-derived molecules (Shigemoto-Mogam et al., 2014). Microglia are also thought to actively contribute to myelination by supplying oligodendrocytes with iron, which is a co-factor that is necessary for myelination (Zhang et al., 2006). The microglial contribution to the differentiation of progenitors into astrocytes and oligodendrocytes should be further explored, because depletion of these types of glial cells are a hallmark of a handful of neurodevelopmental disorders such as schizophrenia and tuberous sclerosis complex (Bauer et al., 2010; Carson et al., 2015).

The complex involvement of microglia does not cease after neurogenesis and gliogenesis, as microglia have also been implicated to be involved in synaptic formation, maturation and maintenance. Early in embryonic development, microglia are the only glial cell type present and growth factors that originate in microglia are known to contribute to neuron survival and to synapse creation (Chamak et al., 1994; Lim et al., 2013; Parkhurst et al., 2013). Recently, more direct evidence has emerged that has shown that microglia contact dendrites, causing an increase in intracellular calcium at that site, which results in an accumulation of actin and eventual synapse formation (Miyamoto et al., 2016). Microglia contact may not be necessary for the initiation of synapse formation nor may it result in the formation of functional synapses, so further investigation is required. However, several findings suggest that microglia do assist in the maturation process of functional neuronal connections. For instance, a microglial fractalkine receptor has been shown to contribute to the development of neural circuitry. In mice that lack this receptor, synaptic connections take significantly longer to establish electrophysiological responses that are typical of mature circuitry (Paolicelli et al., 2011). It is thought that microglia also contribute to synaptic maturation through a transmembrane signaling molecule (DAP12). Hippocampal neurons from mice lacking DAP12 were more electrophysiologically similar to those comprising immature neural networks than to mature ones (Roumier et al., 2004). A later study saw that these mice additionally had many more dysfunctional synapses when they reached adulthood (Roumier et al., 2008). A similar mutation in the human ortholog of the DAP12 gene results in Nasu-Hakola disease, which is a form of dementia (Paloneva et al., 2002). This suggests that microglia are important not only for the formation and maturation of neural circuitry but also for the maintenance of synaptic connections throughout life.

Microglia are always actively monitoring neuronal activity and they use this information to shape neural circuits. This sophisticated role was looked into after the observation that resting microglia have processes that are highly motile as if they are continuously monitoring different regions of their environment (Nimmerjahn et al., 2015). The motility of microglia processes is modulated by level of neural activity. In the developing visual cortex of mice, less active neural circuits have been shown to be populated by microglia with decreased process motility. They were also more frequently found apposed to synapses and tended to accumulate near relatively larger dendrites when compared to control mice (Tremblay et al., 2010). Prior to this observation, microglia were revealed to express many types of receptors including receptors for various neurotransmitters, such as ATP (Pocock and Kenntenmann, 2007). Neurons release ATP when active and low levels of ATP were seen to influence the movement and outgrowth of microglial process (Honda et al., 2001; Dissing-Olesen et al., 2014). However, higher concentrations of ATP will result in the activation of microglial purinergic receptors, which is known to lead to the release of tumor necrosis factor (TNF), a proinflammatory cytokine that is associated with neuronal loss (Hide et al., 2000; Suzuki et al., 2004). Additionally, microglia are equipped with glutamate and opioid receptors and their activation leads to the release of TNF and BDNF respectively (Hagino et al., 2004; Takayama and Ueda, 2005). This is yet another example of the variety of microglial responses that can influence a wide array of different processes.

Moreover, microglia have since been seen to engulf both presynaptic terminals and post-synaptic dendrites in the hippocampus of mice. Mice lacking a microglial fractalkine receptor had significantly more dendritic spines (Paolicelli et al., 2011). These findings provide evidence that microglia engulf synaptic elements and also therefore have a significant role in synaptic

pruning, which is regulated in part by fractalkine receptor activation. A subsequent study showed that microglia-mediated pruning was also activity-dependent. In developing mice, microglia engulfed significantly more presynaptic terminals when the neuronal activity was blocked (Schafer et al., 2012). The functional importance of this activity-dependent pruning process was explored by Parkhurst et al. (2013), who found that induced depletion of microglia resulted in learning deficits. Additionally, they showed that mice that were depleted of microglia had significantly decreased learning-dependent spine formation and elimination. The formation of spines in response to learning was dependent upon the release of BDNF from microglia instead of the activation of the fractalkine receptor that is involved synapse elimination. These results suggest that microglia are largely responsible for activity-dependent remodeling of neural circuitry and are necessary for the cellular changes that allow for learning.

When microglia are activated due to an immune response, such as activation by lipopolysaccharide (LPS) or viral membranes (Gal et al. 2009), they have different effects on their environment. Of most interest is their effect on axon outgrowth and pruning. In the presence of LPS, activated microglia release a repulsive factor that causes the axon growth cone to collapse (Kitayama et al., 2011). Mouse mothers injected with LPS carried embryos with a relatively high proportion of immune activated microglia and a robust decrease in axonal extension in their brains (Squarzoni et al., 2014). Preceding studies had shown that similar exposure during embryonic development gave rise to offspring with profound behavioral abnormalities (Hava et al., 2006; Carpenter et al., 2011). Activation of microglia due to the presence of the West Nile virus has revealed that active microglia prune away many more presynaptic terminals during early postnatal development in the hippocampus of infected mice. Poor performance in learning and memory paradigms indicated that infected mice also had

impaired spatial learning. Another group of mice that were infected with the virus but also genetically manipulated to contain fewer microglia did not have significant presynaptic terminal loss. These results strongly imply that microglia overly prune when immune-activated and that leads to learning deficits. The hallmark pathology of many neurological disorders, including Alzheimer's disease and Down syndrome, is a reduction in the number of synapses or decreased axon extension. Since activated microglia result in decreased axon extension and increased pruning of presynaptic terminals, they should not be ignored when considering the mechanisms that result in such pathologies and cognitive impairments.

An awareness of the myriad roles microglia play in the CNS during development and throughout life makes it evident that slight microglia dysfunction could lead to a wide range of alterations within and between neural networks. Cutting edge research continues to illuminate the potential contributions of aberrant microglia function in neurological disorders. For instance, there are consistent reports of an overabundance of microglia in postmortem autism brains (Morgan et al., 2010). Microglia cell number and activation state are also altered in patients with schizophrenia (Laskaris et al., 2016). An understanding of the contribution of microglia to disorders such as these could lead to a much more effective holistic approach to therapeutically interventions.

The Known Alterations of Glial Function in Neurodevelopmental Disorders:

The orchestration of the development of neural circuitry involves several cell types whose functions must be highly coordinated. Disruption of this functional coordination will lead to improper development of the system. Glial cells are one of the cell types whose presence has been shown to be vital for proper development. Astrocyte dysfunction is known to contribute to neurodevelopmental disorders in a few ways. Abnormal astrogenesis timing leads to a class of

neurodevelopmental disorders that is caused by the disruption of a major signaling pathway that regulates cell proliferation, differentiation and death. Irregular expression of astrocyte-derived factors also contributes to abnormal development by insufficiently supporting dendrite formation and synaptic development and function. The elucidation of specific neuron-astrocyte interactions that are altered in specific neurodevelopmental disorders has made it very evident that a better understanding of the relationship between the two cell types is needed. This understanding would allow for a more comprehensive explanation of how CNS development occurs in healthy individuals as well as what has gone awry in the diseased CNS.

Neurons and astrocytes both originate from the same neuroepithelium, and the time frame in which each cell type arises from neural stem cells is temporally controlled by extrinsic and epigenetic factors (Morrow et al., 2001; Sun et al., 2001; He et al., 2005). A specific protein tyrosine phosphatase (SHP2) is known to activate signaling pathways that promote neurogenesis and decrease production of astrocytes. A mutation in SHP2 that causes constitutive activation of the protein is found in patients with Noonan Syndrome (NS), a neurodevelopmental disorder characterized by learning disabilities as well as various physiological conditions (Tartaglia et al., 2001). This relationship between overactive neurogenic factors and abnormal astrogenesis has been further supported by the decreased astrogenesis that occurs in mice expressing NS-SHP2 (Gauthier et al., 2007). In 2010, a family with high susceptibility to schizophrenia was found to have a mutation in the *Disrupted in schizophrenia 1 gene*, or *DISC1* (Hayashi-Takagi et al., 2010). Prior to this identification, studies had revealed that mice with induced the expression of the mutant *DISC1* gene presented schizophrenia-like behavioral phenotypes (Li et al., 2007) and that the gene's product is involved in neurogenesis (Mao et al., 2009). This knowledge led Wang et al. (2016) to explore the contribution of *DISC1* to astrogenesis and they determined that a

depletion of *DISC1* also results in decreased astrogenesis. SHP2 and *DISC1* regulate astrogenesis through different pathways and mutations in each may lead to a specific neurodevelopmental disorder. This exemplifies the nuanced nature of astrogenesis regulation and how alterations at different points in that regulation can lead to distinct cognitive dysfunction.

Specific cognitive irregularities have also been associated with an abnormal expression of astrocyte-derived factors. Initially, scientists observed that there were altered levels of astrocyte markers in certain brain regions of individuals with neurodevelopmental disorders. For instance, glial fibrillary acidic protein (GFAP) was expressed at a higher level than normal in some brain regions of patients with autism spectrum disorder (ASD) (Laurence et al., 2005). Similarly, there have been reports of a reduction in the expression of phosphorylated GFAP in the frontal cortex of patients with schizophrenia (Webster et al., 2001). More recently, research has begun to unravel how astrocytes could contribute to neuronal dysfunction through expression of specific proteins. One way is through the inability of astrocytes to properly aid in the formation of dendrites. Astrocytes with a mutation in the transcription factor methyl-CpG-binding protein 2 (*MECP2*) have been shown to insufficiently support the formation of dendrites, therefore leading to abnormal dendrite morphology (Ballas et al., 2009). Mutation in this *MECP2* gene causes an X-linked form of ASD known as Rett syndrome. Aberrant spine morphology is also a characteristic of Fragile X syndrome (FXS), another type of X-linked ASD (He et al., 2012). Hippocampal neurons from healthy mice that were co-cultured with FXS astrocytes were characterized by abnormal synapse morphology. These cultures were determined to have a significant reduction in thrombospondins, extracellular matrix proteins that are known to be important for astrocytic support of synapse formation (Christopherson et al., 2005; Cheng et al., 2016). Introduction of thrombospondins into these cultures prevented aberrant synapse

formation, which further demonstrates that importance of astrocyte-derived factors in the proper development of neural circuits.

Myelin is another aspect of neural circuitry that, if altered, leads to improper cognitive function. There have been a handful of observations of reduced myelination of certain tracts in the brains of patients with a neurodevelopmental disorders. Abnormalities in myelination levels were revealed by MRI analysis of whole brains of children with ASD (Courchesne et al., 2001). Oligodendrocytes in some brain regions of patients with ASD had altered levels of oligodendrocyte-specific gene expression (Zeidán-Chuliá et al., 2015), which could account for the increased myelination that has been observed in the cerebrum and the cerebellum of children with ASD (Hendry et al., 2006). In adults with ASD, other brain regions, such as the amygdala, have more recently been revealed to have significantly fewer oligodendrocytes when compared to age-matched controls (Morgan et al., 2014). It is currently unknown what causes abnormal myelination in autism, but new findings are illuminating the genetic cause of oligodendrocyte dysfunction in other neurodevelopmental disorders. The hypomethylation that is characteristic of the brains of patients with Down syndrome was determined to be a result of decreased oligodendrocyte differentiation. Transcriptome analysis found that a family of co-expressed genes important for normal neural development is downregulated in DS brains. About half of these co-expressed genes are highly expressed in oligodendrocyte lineage cells, a few of which are known to be important for myelin formation, such as myelin-associated glycoprotein and myelin basic protein (Olmos-Serrano et al., 2016). This study further showed that this hypomethylation results in slower axonal conduction within the neocortex, likely due to specific changes in oligodendrocytes. Early intervention that is aimed at increasing myelination may lessen the neurological and cognitive symptoms caused by DS. This intervention method should

also be considered when developing therapies for other neurodevelopmental disorders with aberrations in myelination.

In some neurodevelopmental disorders, a loss of myelin is due to an over abundance of immune active microglia. One such disorder is periventricular leukomalacia (PVL), which is considered to be a white matter disorder. PLV commonly leads to the development of cerebral palsy, a movement disorder that may also be accompanied by learning and communication deficits (Liu et al., 2013). Early stages of PVL in neonatal brains are characterized by increased microglia proliferation and activation, which causes a drastic increase in TNF-alpha (Kadhim et al., 2001). This cytokine has long been known to lead to the degeneration of myelin and the death of oligodendrocytes (Selmaj and Raine, 1988). Together, this strongly relates an overabundance of immune-active microglia to the decrease in white matter that is characteristic of PVL. Aberrant microglial activation has also been implicated as a contributor to the overgrowth and dysfunction of various regions in autistic brains. Whole brain quantification has revealed that autistic brains have many more microglia than controls, but regional analysis has shown that some regions have more microglia than average and others have fewer than average (Morgan et al., 2014). Post mortem analyses have consistently reported a decrease in neuronal density in the cerebellum of ASD patients, which is where there is a prominent increase in microglia activation (Vargas et al., 2004). This study also showed that there was an overabundance of microglial-derived cytokines throughout the brain tissue of the ASD brains. The prevalence of one cytokine was increased only in the frontal cortices of autistic brains. This specific cytokine regulates neuronal differentiation and growth. This is of interest because the developing frontal cortex of children with ASD is characterized by neuronal overgrowth and disorganization (Stoner et al., 2014). These somewhat contradictory effects of microglia cytokine

production in the autistic brain are yet another illustration of how nuanced microglia-neuron interactions are within the CNS. Moreover, they argue in support of the idea that microglia involvement must not be forgotten when considering the molecular and cellular mechanisms underlying the diseases that cause altered CNS development.

These are only a handful of examples of abnormalities of glial cell function discovered in neurodevelopmental disorders. There are a myriad of other findings that link the three glial cell types to atypical neurodevelopment (see reviews: Reemst et al., 2016; Yamamuro et al., 2015). The realization that glial cells are active modulators and support cells of neural circuitry has led researchers to study these cells more closely in an attempt to understand their widespread functions in both health and disease. It will be this research that illuminates the details of how glial cells contribute to neurodevelopmental disorders. Once this understanding is obtained, comprehensive therapeutic interventions can be developed either to aid in proper neural development or to lessen the symptoms caused by the abnormal development of the CNS.

Potential Contributions of Glia to Neurodevelopmental Disorders

In recent years, it has become clear that glial cells are more involved in the development of the CNS than was previously appreciated. This new understanding makes it imperative that the study of neurodevelopmental disorders include a glial, as well as neuronal, perspective. Many of the pathologies that are characteristic of neurodevelopmental disorders could be caused by as yet unexplored glial-cell dysfunction. The altered neural circuitry in individuals with ASD, Down syndrome, attention-deficit/hyperactivity disorder, and Tourette syndrome, for instance, could well result from changes in glial biology.

Autism-Spectrum Disorder (ASD)

The neuropathological phenotype of ASD is ill defined, due to the immense variability between individuals on the spectrum. However, patients with ASD commonly present with macrocephaly, abnormal neuron size and organization, increased immune activation, and altered myelination patterns. Each of these alterations could as likely arise from disturbed glial cell mechanisms as from neuron-specific disturbances.

For instance, the macrocephaly that is common in ASD (as well as other developmental disorders; Fidler et al., 2000) is likely to be due to an increased number of neurons in the cortex. Postmortem analysis of 7 autistic male brains revealed a 67% increase in neuron number in the prefrontal cortex of these brains when compared to control brains (Courchesne et al., 2011). This drastic increase may indicate an upregulation of neurogenesis during prenatal brain development. Such upregulation could result from an increase in pro-inflammatory cytokines, such as interleukin-6 (IL-6) and TNF-alpha (seen in postmortem tissue from autistic brains: Li et al., 2009; Wei et al., 2011), which are produced by both astrocytes and microglia. IL-6 has been shown to induce the differentiation of neural stem cells into excitatory neurons (Islam et al., 2009; Erta et al., 2012).

In addition to increased neuron number, the size and organization of neurons in certain brain regions of individuals with ASD is often abnormal. In some regions of autistic brains, such as the anterior cingulate gyrus, the neurons are smaller than average and packed more densely. In other regions, such as in the cerebellar nuclei and inferior olive, the neurons are unusually large (Bauman and Kemper, 1994; Kemper and Bauman, 1998). The size of neurons is known to be influenced by neurotrophins, such as neuron growth factor (NGF); in low concentrations of NGF, neuron size is decreased (Franklin and Johnson, 1998). Astrocytes are known to release

several neurotrophic factors, including NGF (Furukawa et al., 1986), and recently, researchers have discovered that the astrocytic production of NGF is up-regulated when astrocytes are in their immune-activated state (Sáez et al., 2006). This is interesting because in the brains of individuals with ASD, there is an elevated immune response (Li et al., 2009). An increased proportion of immune-active astrocytes in autistic brains would result in an over-abundance of NGF in some brain regions, which could cause some subsets of neurons to get bigger than normal. Other growth factors (i.e. epidermal growth factor, EGF) are significantly decreased in the CNS of individuals with ASD [ref.?]. This could account for the decreased neuronal size in other regions of autistic brains.

The increased immune response that is common in autistic brains specifically involves increased presence of immune-active microglia and reactive astrocytes (Vargas et al., 2005). This enhanced immune response is likely what causes the increase in pro-inflammatory cytokines that has been detected in post-mortem tissue and blood from patients with ASD (Jyonouchi et al., 2001; Wei et al., 2011). As previously discussed, the immune response within the CNS is a highly regulated process throughout development. Additionally, microglia and astrocytes play important roles in removing foreign or degenerating material from the CNS to prevent an unnecessary immune response (Serhan and Savill et al., 2005). If the clearance efficiency of microglia or astrocytes is compromised, then the CNS is more vulnerable to an enhanced immune response that could lead to neural dysfunction. Altered immune response has been considered as a contributor to ASD and an interesting correlation has come to light: children with ASD commonly have mothers with chronic inflammation, either from autoimmune diseases or infections (Keil et al., 2010). Animal studies have supported that prolonged activation of a mother's immune system can alter the developmental trajectory of the offspring's CNS

(Hava et al., 2006; Carpenter et al., 2011; Squarzoni et al., 2014). The molecular and cellular changes that accompany reactive gliosis have not yet been characterized, but the devastating changes that can accompany enhanced immune responses during development are strong indicators that various mechanisms are being disturbed.

If more microglia and astrocytes are in their immune-active state, then it is likely that fewer of these glial cells are present in the developing CNS in their neutral state. This is potentially problematic because neutral glial cells contribute to important processes such as modulation of the maturation and pruning of neural circuitry. One wonders whether suppression of a mother's immune response during the period in gestation in which the blood-brain barrier is slightly permeable could possibly decrease her offspring's risk of ASD and other neuropsychiatric disorders.

White matter within the CNS has also been seen to be abnormal in some individuals with ASD. The amount of white matter is increased and certain cortico-cortical tracts are hypermyelinated (Barnea-Goraly et al., 2004; Herbert et al., 2004). One may ask: does the increase in myelination result from an abnormality in oligodendrocytes? Very little research has been done to address this question, but just last year, it was found that various oligodendrocyte-specific markers, such as *OLIG1, OLIG2* and *MBP*, are upregulated in some regions of the brains of ASD patients (Zeidán-Chuliá et al., 2016). Interestingly, one genetic cause of ASD is Fragile X syndrome (FXS),which results from a mutation that prevents the production of Fragile X Mental Retardation Protein (FMRP), a local protein synthesis regulator that represses the translation of its mRNA targets until the protein is needed (Laggerbauer et al., 2001). Myelination abnormalities are also associated with FXS (Barnea-Goraly et al., 2003; Wang et al., 2004), and FMRP is found in oligodendrocyte precursor cells as well as in mature

oligodendrocytes (Giampetruzzi et al., 2014). Moreover, the mRNA for MBP, which is necessary for myelin formation, is known to be under the translational regulation of FRMP (Wang et al., 2004). Thus, the lack of regulation of MBP production in FXS could, in theory, result in an over-abundance of myelin and this could be what influences the premature myelination and the overall hypermyelination of the autistic CNS. The quickened cortical and interhemispheric signaling that would result from increased myelination could possibly allow for the augmented cognitive abilities that some ASD patient's have and could also contribute to the aberrant signaling that would cause cognitive dysfunction.

Down Syndrome (DS)

Down syndrome (DS) is another neurodevelopmental disorder. Like FXS, it is genetically caused. It results from the presence of three copies of the 21st chromosome and individuals with this disorder often have intellectual disability (Korenburg et al., 1994). The brains of individuals with DS are characterized by decreased neurogenesis (Guidi et al., 2008), decreased brain size (Wisniewski et al., 1990), and altered synaptic function (Belichenko et al., 2007). Each of these abnormal pathologies could involve glial cells, and exploration of specific functional irregularities in patients with DS might well reveal that glial cells contribute to this neurodevelopmental disorder more than currently assumed.

The decreased neurogenesis that occurs in the brains of patients with DS may arise from a multitude of mechanisms. One very likely mechanism is through a decrease in astrocyte-released factors that are known to promote neurogenesis. A recent study by Chen et al. (2013) has explored the influence of astrocytes on neurogenesis in DS by culturing DS neural progenitor cells (NPC) with either DS astrocyte-conditioned medium (ACM) or control ACM. They found that the NPCs cultured in DS ACM gave rise to significantly fewer neurons and more astrocytes

than did those cultured in control ACM. To test whether this was due to the increased expression of S100-beta, an astrocyte-derived growth factor, they cultured NPCs in both DS ACM and S100-beta-silencing RNA, and found an increase in neurogenesis back to about wild-type levels. Other factors likely play a role in neurogenesis, but this study alone suggests that astrocytes contribute to the decreased neurogenesis in DS brains.

The presence of fewer neurons is likely a major contributor to the overall small brain size of DS patients. Smaller numbers of neurons could result from either decreased neurogenesis or increased cell death. Apoptotic neuronal cell death is known to be induced by elevated levels of reactive oxidant species (Cheng et al., 2001), such as nitric oxide (NO), and elevated levels of these types of molecules have been found in cultures of cells from DS brains (Busciglio and Yankner, 1995). Interestingly, activation of S100-beta has been shown to result in an increase in NO production (Hu et al., 1996). Chen et al. (2013) looked at this relationship and they found that cell death was induced in cultured 6- to 7-week-old DS neurons when DS ACM was added to the culture. Together, these two findings suggest a connection between the over expression of S100-beta in DS and an increase in production of NO that causes apoptosis. S100-beta is just one glial factor that has so far been identified to play a role in the decreased brain size of DS patients, but it seems likely that there are a myriad of other glia-neuron interactions that are altered due an over-abundance of the genes present on the 21st chromosome.

Astrocytes are important in neuronal maturation even after early differentiation, and astrocyte-derived factors have been identified as pertinent elements for the formation of functional synapses (Clarke and Barres, 2013). Chen et al. (2013) once again used their DS neuron and DS ACM paradigm to explore the differences in the electrophysiological properties of DS neurons when grown in cultures without any ACM, with DS ACM, and with control

ACM. DS neurons that were cultured with control ACM were electrophysiologically similar to control neurons; but DS neurons that were grown in DS ACM exhibited similar electrophysiological characteristics to those that were cultured without any ACM at all. This revealed that DS astrocytes fail to provide maturing neurons with the factors that were necessary for them to develop electrophysiological properties typical of mature wild-type neurons.

The function of mature DS neurons may also be altered by microglia-derived factors, some of which are known to be up-regulated in DS. One of these factors, Interleukin-1, is a cytokine produced by microglia during an immune response (Dinarello et al., 1988); it modulates neuronal events such as long-term potentiation (Murray and Lynch, 1998). In the CNS of DS patients, there is an increased in the number of immune-active microglia (Griffin et al., 1989). Thus, in DS, the ability of astrocytes to properly support maturing neurons is compromised in some currently unidentified way, and the increased microglia activation potentially results in impaired long-term potentiation. Both of these findings support the notion that glial cells play a role in the impaired cognitive function exhibited by individuals with DS.

Attention Deficit/Hyperactivity Disorder (ADHD)

A different type of cognitive dysfunction occurs in individuals with attention deficit/hyperactivity disorder (ADHD). Though there is wide variability between individuals with this disorder, the majority of them present with impaired attentional control, cognitive hyperactivity, and behavioral impulsivity (Castellanos and Tannock, 2002; Klein et al., 2006). There is no clear, consistently observed pathology associated with ADHD, and therefore a detailed discussion regarding the possible influence of glial cells on the disease pathology would be undirected. However, a perplexing feature of this disorder is that the severity of each of these

phenotypes can differ within individual subjects across trials. The underlying neurological mechanisms that permit high intrasubject variability are still largely unknown; however, consideration of glial cell involvement may aid in the explanation of this phenomenon and neuron-glia interactions can be explored.

The prevailing hypothesis in regard to fluctuations in the performance of ADHD patients in cognitive and attention tasks is one pertaining to energetics. This hypothesis is built upon the idea that rapidly firing neurons that are continually active during working memory tasks quickly deplete their ATP stores and depend upon glial support for additional metabolites. If glial cells do not continuously provide the neuron with the metabolites that are necessary for repetitive activation, then the activation of neural circuitry will decrease. Several experts on this topic believe that this could account for the inefficient and inconsistent cognitive performance of individuals with ADHD (Russell et al., 2006). An obvious possible abnormality that would hinder metabolite exchange would be a decrease in either neuronal or glial cell lactate or glucose transporters. The only quantification of lactate or glucose transporter expression in ADHD brains concluded that astrocyte-specific lactate and glucose transporters were up-regulated in a few brain regions and that a neuron-specific lactate transporter was up-regulated in some regions, but down-regulated in the prefrontal cortex and the cerebellum (Hefte et al., 2012). These results do not expressly indicate that astrocytes are insufficient providers of metabolites, but this does not liberate them from possible dysfunction in regard to metabolic support of neural circuitry.

Another mechanism that may be occurring in the brains of patients with ADHD is one that is targeted by a treatment that has been in use for the last 27 years. Alpha-2 adrenergic receptor agonists are a class of medications used to treat ADHD. Although the exact mechanism by which this drug works to relieve ADHD symptoms is not known, the drugs are thought to

work by modulating neurotransmission, specifically by interacting with neuron-specific alpha-2 adrenergic receptors. Suprisingly, however, it was shown that an agonist specific to this receptor increased astrocytic disposal of glutamine, an important precursor to glutamate (Huang et al., 2000). This finding indicated that either alpha-2 adrenergic receptor activation leads to some sort of signaling that influences astrocyte glucose metabolism or the agonists also can activate the beta-2 adrenergic receptors that are expressed by astrocytes. These astrocyte adrenergic receptors are known to influence astrocytic glucose uptake through GLUT1, as well as the speed in which glycogen is broken down (Gibbs et al., 2008). The fact that ADHD drugs within this class can affect glucose uptake and metabolism within astrocytes further supports the notion that there is an imbalance between metabolic needs of neural circuits and the rate in which astrocytes can provide these circuits with metabolites. Metabolic irregularities are becoming increasingly recognized as contributors to cognitive dysfunction. Therefore, even though many of the possible aforementioned mechanisms are poorly supported and even more poorly understood in the context of ADHD, this hypothesis should be considered in future explorations aimed at understanding the inner workings of this disorder.

Tourette Syndrome (TS)

Not all neurodevelopmental disorders manifest themselves in cognitive dysfunction. Some result in motor dysfunction instead. For instance, Tourette syndrome (TS) is a neurodevelopmental disorder that results in involuntary movements and vocalizations (Kurlan et al., 2010). The most prominent pathology associated with this disorder is neuronal loss and changes in the circuitry of the basal ganglia, a region known for its involvement in motor control (Leckman et al., 2010). Knowing that astrocytes and microglia both perform irreplaceable roles in the formation of proper connectivity within neural networks, it would seem reasonable to

consider these cells when attempting to understand how basal ganglia connectivity has been compromised in TS. This realization, however, did not seem to inspire researchers to look into the involvement of glial cells in TS. Instead, it was the similarity between TS and an autoimmune disease known as PANDAS, which is characterized by TS-like symptoms, that led researchers to look into the immunological etiology of TS. There has been substantial evidence of an increased inflammatory response in individuals with TS as proven by increased levels of pro-inflammatory cytokines, including IL-1 and TNF-alpha (Leckman et al., 2005; Cheng et al., 2012). It was not until more recently that regional microglia population differences in TS were explored in order to determine the source of increased cytokine production. Lennington et al. (2016) did post-mortem analysis of TS brains and described an increased microglia presence in the striatum within the basal ganglia. These microglia were observed to have a morphology that is consistent with activation due to neurotoxicity. Other studies have shown increased microglia activation in vivo within other portions of the basal ganglia (Kumar et al., 2015). An increased presence of certain cytokines originating from immune-active microglia leads to increased neuronal cell death (Li et al., 2014; Neniskyte et al., 2014). Taken together, these findings potentially link the over-activation of microglia with the neuronal loss that is seen in the basal ganglia of individuals with TS. However, this connection does not address the question: how could increased microglia activation lead to altered basal ganglia connectivity?

Microglia are important for the maturation and proper pruning of neural circuitry. This is made evident through the aberrant circuitry that arises in animals devoid of specific microglial fractalkine receptors (Paolicelli et al., 2011). Unpublished work by Frick et al. has yielded results from a mouse model of TS that suggests that these mice have deficiencies in a specific microglial fractalkine receptor. These preliminary results coincide with other findings that show that a

deficiency in this same microglial fractalkine receptor results in an abnormal microglia morphology that is also seen in the mouse model of TS (Pagani et al., 2015). Irregular expression of this type of receptor on microglia would theoretically result in abnormal connectivity due to insufficient support and pruning during development. This may not be the exact mechanisms in which microglia activation result in the changes seen in the basal ganglia of individuals with TS, but these are example of the ways in which the disorder can be looked at from the perspective of altered glial cell function.

Accepting that glial cells likely play a role in various cognitive and motor dysfunctions is likely to lead to a more nuanced – and more complete – understanding of the cellular as well as molecular mechanisms that have gone awry during development to give rise to neurodevelopmental disorders such as the ones just discussed. It could very well be that a more integrated neuron/glia approach will move to the forefront of the field of neurodevelopmental research in the near future.

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