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Review

Glia: victims or villains of the aging brain?

Isabel H. Salas, Jillybeth Burgado, Nicola J. Allen*

Salk Institute for Biological Studies, Molecular Neurobiology Laboratory, 10010 North Torrey Pines Road, La Jolla, CA 92037, USA

ABSTRACT

Aging is the strongest risk factor for metabolic, vascular and neurodegenerative diseases. Aging alone is associated with a gradual decline of cognitive and motor functions. Considering an increasing elderly population in the last century, understanding the cellular and molecular mechanisms contributing to brain aging is of vital importance. Recent genetic and transcriptomic findings strongly suggest that glia are the first cells changing with aging. Glial cells constitute around 50% of the total cells in the brain and play key roles regulating brain homeostasis in health and disease. Their essential functions include providing nutritional support to neurons, activation of immune responses, and regulation of synaptic transmission and plasticity. In this review we discuss how glia are altered in the aging brain and whether these alterations are protective or contribute to the age-related pathological cascade. We focus on the major morphological, transcriptional and functional changes affecting glia in a range of systems, including human, non-human primates, and rodents. We also highlight future directions for investigating the roles of glia in brain aging.

1. Introduction

As we age, our brains experience broad alterations that result in a progressive decline of functional capabilities. Physiological brain aging is associated with changes in brain size, vasculature, neuronal connections and networks (Palmer and Ousman, 2018). These aging-induced changes frequently materialize as deficits in cognitive abilities, sensory perception, and motor function and coordination. On a macroscopic level, human brain experiences a reduction in grey and white matter volumes and subsequent enlargement of ventricles (Drayer, 1988; Jack et al., 1997). On the cellular and molecular levels, some hallmarks of brain aging are mitochondrial dysfunction, increased oxidative stress and damage, metabolic alterations, autophagy dysfunction, and dysregulated stress and inflammatory responses (Mattson and Arumugam, 2018). In addition, mitotic cells also undergo a process called senescence, characterized by telomere shortening and an arrest of the cell cycle (Childs et al., 2014), although whether brain cells experience cellular senescence is still under discussion (Chinta et al., 2015).

Aging is also linked to an increased risk for developing a variety of different diseases including stroke, white matter lesions, vascular dementia and neurodegenerative diseases such as Parkinson's and Alzheimer's disease (Wyss-Coray, 2016). However, the mechanisms that predispose the aged brain to neurodegeneration and vascular alterations are still not fully understood (Chakrabarti and Mohanakumar, 2016).

In the past, most research into aging-related alterations in the brain focused on neurons. Nonetheless, non-neuronal glial cells are the first cells responding to "stress" in the central nervous system (CNS) (Palmer and Ousman, 2018). The major types of glial cells in the CNS are astrocytes, microglia, and oligodendrocytes. Astrocytes are star-like cells that interact closely with synapses, blood vessels, and other glial cells, regulating synapse formation and function and providing nutritional support to neurons (Allen, 2014). Microglia are the resident immune cells in the CNS, and their main function is to survey the brain environment and respond to injury and disease (Salter and Stevens, 2017). In turn, oligodendrocytes are the primary cell type responsible for myelination of neuronal axons in the CNS. Their ability to properly ensheath axons is critical for neuronal function (Young et al., 2013). These diverse and critical functions performed by the different glial cells situate them in a position to be affected by brain aging. Indeed, a transcriptional study from human aging brain showed that glial cells, and not neurons, displayed the majority of differential gene expression with aging (Soreq et al., 2017).

The homeostatic role of glial cells is tightly linked to their protective capacities (Allen and Lyons, 2018). If glia show vulnerability to brain aging, this may also affect their homeostatic functions, decreasing neuroprotection and further contributing to age-associated pathological alterations (Rodríguez-Arellano et al., 2016). Additionally, with aging, glial cells may upregulate pathways that negatively impact neuronal and synaptic function, so actively contributing to the damaging changes that occur with age (Boisvert et al., 2018; Clarke et al., 2018). There are also evidence pointing towards signs of cellular senescence in glia cells (such as telomere shortening), which might also interfere with their physiological function. However, most of this evidence for cellular senescence have only been shown in culture (Flanary and Streit, 2004).

* Corresponding author.
E-mail address: nallen@salk.edu (N.J. Allen).

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Table 1
Summary of what is considered aged for the different models discussed.

Animal model	Age
Mouse	> 18 months
Rats	> 22 months
Rhesus monkeys	> 22 years
Humans	> 65 years

Hence, understanding how glia are altered in aging and the functional consequences of these alterations on brain function are essential first steps to discern the mechanisms occurring in brain aging and contributing to aging-related diseases. In this review we discuss the specific changes occurring in each type of glial cell with aging, and the consequences of these alterations on brain function. We discuss data from humans, animal models and *in vitro* studies. In table 1 we summarize what we classify as age for the different models discussed. Numerous research on aging has also been done in different model organisms including Drosophila and *C. elegans* (Davie et al., 2018; Frakes et al., 2020), but these studies will not be discussed as it goes beyond the scope of this review.

2. Astrocyte alterations with aging

2.1. Astrocyte properties

Astrocytes are star-shaped glial cells that comprise around 20% of brain cells, with variations depending on the CNS region (von Bartheld et al., 2016). They tile the entire CNS, contacting synapses, blood vessels and other glial cells with their fine processes (Sofroniew and Vinters, 2010). Astrocytes play essential roles regulating neuronal function. During development they release synaptogenic signals to promote synapse formation and can also regulate synapse elimination (Allen et al., 2012; Chung et al., 2013; Farhy-Tselnicker et al., 2017). In the adult brain, astrocytes regulate synaptic function forming part of the tri-partite synapse, recycling neurotransmitters, buffering extracellular potassium, and releasing gliotransmitters, although the mechanisms responsible for this release are still not clear (Hamilton and Attwell, 2010; Savtchouk and Volterra, 2018). They are also structural and functional components of the blood brain barrier, regulating blood flow and providing metabolic support to neurons (Allen, 2014). Similar to microglial cells, astrocytes become 'reactive' in response to CNS insults (Burda and Sofroniew, 2014). Astrocyte reactivity is characterized by an increased expression of the glial fibrillary acidic protein (GFAP) and changes in morphology and function (Wilhelmsson et al., 2006). The process of becoming reactive is gradated and may vary from subtle changes to a deep irreversible alteration, depending on the context (Anderson et al., 2014). As such it is not easy to predict whether astrocyte reactivity is beneficial or detrimental to the surrounding cells. Astrocytes, like neurons, are present in the brain throughout life. They are not replenished, nor divide except in the case of injury. Thus, they are particularly susceptible to age associated dysfunction such as accumulation of a lifetime of stress (Cohen and Torres, 2019).

2.2. Do astrocytes become reactive with aging?

To what degree astrocytes react to brain aging is a fascinating question that is still understudied. As mentioned before, astrocyte reactivity is a continuum process that is constantly evolving depending on the context. Thus, defining the hallmarks of "astrocyte reactivity" perse, is not simple.

2.2.1. Changes in GFAP

One readout widely used to quantify astrocyte reactivity is the increase of GFAP levels. One concern about this approach is the fact that basal expression of GFAP is variable depending on the brain region, i.e.: hippocampus shows very high expression while cortex almost none (Kimelberg, 2004). There are also technical differences depending where and how GFAP is measured in each study: while some characterized number of GFAP-positive cells (Diniz et al., 2010), others quantified GFAP intensity (Kanaan et al., 2010), area (Rodríguez et al., 2014) or mRNA levels (Nichols et al., 1993). This is very important, as GFAP mRNA levels are fairly high in astrocytes in all brain regions, and it is the protein levels that differ basally (Hol and Pekny, 2015). All these variables can lead to conflicting results making the interpretation challenging.

For example, an early characterization from the 1990's reported that GFAP mRNA levels were increased with age in human and rat hippocampus (Nichols et al., 1993). These findings were consistent with another study showing a correlation between aging and GFAP protein levels in the human hippocampus and entorhinal cortex (David et al., 1997). Interestingly, this correlation was less robust in other brain areas such as the isocortex. In turn, aged rhesus monkeys (22 years old) displayed an increase in GFAP-positive astrocyte number and fluorescence intensity in the cortex, putamen and hippocampus compared to young monkeys (9 years). However, other brain areas including the substantia nigra and ventral tegmental area showed no changes in GFAP with age (Kanaan et al., 2010).

Regional differences in astrocyte GFAP alterations with aging are also evident in mice: Rodriguez et al. showed an increase in GFAP surface area and volume in individual astrocytes with aging in mouse hippocampal regions (CA1 and dentate gyrus), while in the entorhinal cortex GFAP was decreased (Rodríguez et al., 2014). Similarly, Diniz et al. observed an increased number of GFAP-positive astrocytes in aged mice (20 months) compared to adult (6 months) in the polymorphic layer of the dentate gyrus, but not in the granular layer (Diniz et al., 2010).

These results suggest that aging does not affect astrocytes equally in all brain regions. However, as mentioned above, every study used different readouts to measure GFAP which can explain the high variability of the results. Additionally, it is important to note that each study characterized different species, and even within the same specie (i.e.: mouse), differences in the genetic background and environmental exposure can have an important impact on the aging phenotype (Neuner et al., 2019).

2.2.2. Other ways to measure astrocyte reactivity: transcriptional changes

Astrocyte reactivity is not only characterized by an increased expression of GFAP, but also by an upregulation of different markers such as pro-inflammatory signals and the complement pathway (Liddelow and Barres, 2017; Sofroniew and Vinters, 2010). In recent years, development of next generation sequencing techniques have made it possible to obtain a broader characterization of transcriptional changes occurring in astrocytes and other cell types in the aging brain.

Thus, to further investigate whether astrocytes show differential spatial vulnerability to aging, two recent reports characterized the transcriptional profiles of astrocytes in multiple brain regions of adult and aged mice using RNA-sequencing (Boisvert et al., 2018; Clarke et al., 2018). Both studies provided evidence that aged astrocytes develop an overall partially reactive-like phenotype, characterized by an increased expression of complement pathway and neuroinflammatory genes in all brain regions. Interestingly, astrocytes isolated from the hypothalamus, cerebellum, hippocampus, and striatum experienced greater remodelling of their transcriptome when compared to cortical astrocytes (Boisvert et al., 2018; Clarke et al., 2018). These results illustrate the differential susceptibility of particular brain regions to aging-induced alterations in astrocyte gene expression.

Similarly, analysis of 10 different cortical and subcortical brain regions from young, middle-age, and old human individuals (> 75 years old) demonstrated that astrocyte gene expression profiles change

differently across age and brain region (Soreq et al., 2017). The most complex shift was prominently observed in the hippocampus and substantia nigra. A different meta-analysis of human transcriptome data from four studies also suggested that aged astrocytes in the human prefrontal cortex exhibit a reactive phenotype (Payán-Gómez et al., 2018).

In summary, these results support that with aging astrocytes develop a partially reactive phenotype that varies in different brain regions. This regional variability might contribute to the differential regional vulnerability to aging and age-related neurodegenerative disorders. However, whether age-associated astrocyte alterations are detrimental or protective for neuronal viability is still a matter of discussion. On the one hand, human data suggest that astrocytes seem to be more susceptible to aging-induced alterations in brain regions more vulnerable to synapse loss and Alzheimer's disease neurodegeneration such as hippocampus and entorhinal cortex, compared to isocortex (David et al., 1997; Soreq et al., 2017). Mouse studies however, revealed the biggest gene expression changes in astrocytes in the cerebellum, a region mostly spared in Alzheimer's and Parkinson's disease pathology (Boisvert et al., 2018). These data support that astrocyte reactivity might be protective for age-associated neurodegenerative disorders.

Interestingly, a single cell sequencing analysis of hippocampal astrocytes in a mouse model for Alzheimer's disease revealed the presence of a disease-associated astrocyte cluster. This cluster was also present in aging mouse and human astrocytes, suggesting a similar astrocyte phenotypic state switch occurs in normal aging and Alzheimer's disease in the hippocampus (Habib et al., 2020). More research is needed to understand how and whether astrocyte changes with aging predispose the brain to neurodegenerative diseases. This is a complicated process that might vary between individuals, brain regions and exposure to different genetic and environmental risk factors (Chouliaras et al., 2010). In addition, astrocyte responses to aging and neurodegeneration are likely affected by crosstalk with other cell types including neurons, microglia and oligodendrocytes (Palmer and Ousman, 2018).

2.3. Does astrocyte morphology change with aging?

In response to CNS insults, besides changing their gene expression, astrocytes can also alter their morphology (Ferrer, 2017). Whether and how astrocyte morphology is affected in the aging brain is still a matter of discussion.

A standard method to characterize changes in astrocyte morphology is the use of antibodies against GFAP for immunohistochemistry studies. It is important to mention that the use of GFAP staining to measure astrocyte complexity is limited. GFAP is a fibrillary protein that binds to intermediate filaments of the cytoskeleton, thus, changes occurring in the fine processes cannot be detected using this marker. Additionally, as mentioned before, GFAP expression is tightly linked to astrocyte reactivity and alterations in the size of GFAP-positive processes can be explained by an increased accumulation of GFAP, while the overall size and complexity of the astrocyte may not be altered. Using this approach, one group showed an increased area and complexity of GFAP-positive astrocytes in the hippocampus, but not in the entorhinal cortex, of 24 month-old mice compared to 3 month-old (Rodríguez et al., 2014). Other groups however, support that with aging, rat hippocampal astrocytes become fewer, smaller, with thicker and shorter branches (Cerbai et al., 2012). Macaque astrocytes also show a decreased complexity (determined by the number of branches) in the frontal lobe (Robillard et al., 2016).

The limitation of using GFAP to determine astrocyte size and structure can be overcome using different techniques such as intracellular dye filling or the analysis of cytoplasmic markers that fill all astrocyte processes. For example, Grosche *et al.* characterized individual astrocyte territories in mouse neocortex and hippocampus using Golgi impregnation. This method was compared to dye filling techniques and yielded comparable results. The study found an age-dependent increase in astrocyte territories in 21-month-old mice compared to 5-month-old. Cortical, but not hippocampal astrocytes also showed increased branching with aging. In both regions, the increased astrocyte area was accompanied by a substantial increase in the overlap of astroglial domains (Grosche et al., 2013). A different study used s100b to mark astrocyte cytoplasm and also observed a significant increase in astrocyte volume in the dentate gyrus and entorhinal cortex, but not in the CA1 region, in 24 month-old mice compared to 3 month-old (Rodríguez et al., 2014).

Taken together, these experiments suggest that astrocytes respond to brain aging by changing their morphology, increasing their volume and complexity. These alterations seem to occur in a region-specific manner. However, it is difficult to extrapolate general conclusions from these studies as each one used different methods to identify and quantify astrocyte morphology, different animal species and analysed different brain regions. More systematic methods of morphological characterization of astrocytes across different species and brain regions using dye filling techniques or astrocyte-specific expression of fluorescent reporter proteins will benefit the field in order to address these relevant questions.

2.4. Do astrocyte numbers change with aging?

In the adult brain astrocyte proliferative capacities are extremely limited, although in certain contexts, such as brain trauma, astrocytes can proliferate to generate a protective scar (Liddelow and Barres, 2017). For years it was believed that astrocyte number was increased in the aging brain (Diniz et al., 2010; Hansen et al., 1987; Wang et al., 2006). However, these studies used GFAP to mark astrocytes, and as mentioned not all astrocytes express GFAP under physiological conditions (Hol and Pekny, 2015). Thus, an increase in GFAP-positive astrocytes more likely reflects an increase in astrocyte reactivity, but not an absolute increase in astrocyte numbers. Indeed, when using different astrocytes markers, such as s100b, no differences in cell number were observed in mouse neocortex and CA1 region in old mice (21 month-old) compared to adult ones (5 month-old) (Grosche et al., 2013). Similarly, electron microscopy studies in humans revealed no differences in the number of astrocytes associated with age (Fabricius et al., 2013; Pelvig et al., 2008). However, it is important to mention that cell identification in the latter studies was done using only morphological features, which may not always be accurate. A more recent study using single-cell transcriptomics also suggests there is no major change in astrocyte cell number with aging (Ximerakis et al., 2019). In summary, from these experiments we can conclude that brain aging is not accompanied by significant changes in the number of astrocytes.

2.5. Are astrocyte functions altered with aging?

Astrocytes play key roles in regulating neuronal functions that are compromised in aged brains, such as regulating synaptic plasticity and forming part of the blood brain barrier structure (Erdő et al., 2017; Matias et al., 2019). It is then worth asking whether changes in astrocytes underlie the functional alterations observed in aged brains.

2.5.1. In vitro approaches

One approach that researchers have taken to study changes in astrocyte function with aging is the use of *in vitro* astrocyte cultures. Neonatal rodent astrocytes maintained *in vitro* for several weeks display signs of stress such as increased β-galactosidase and GFAP expression (Kawano et al., 2012; Pertusa et al., 2007), providing an *in vitro* model to characterize functional changes of astrocytes with aging. However, these models have several limitations (discussed below). These studies suggest that aging astrocytes have a toxic effect on neuronal survival and function. Pertusa and colleagues reported increased generation of reactive oxygen species in astrocytes cultured for 90 days compared to

astrocytes cultured for only 10 days *in vitro*. In addition, when co-cultured with neurons, aged astrocytes displayed a reduced ability to maintain neuronal survival compared to young ones (Pertusa et al., 2007). These results are in line with a separate study that co-cultured neonatal cortical mouse astrocytes maintained in culture for 9 weeks with hippocampal neurons and showed a reduction in the pool of readily releasable synaptic vesicles. Interestingly, other neuronal features such as synaptogenesis and synaptic transmission were not affected by astrocyte aging using these models (Kawano et al., 2012).

Although useful, these "aging in a dish" models have some limitations, including that astrocytes are isolated at neonatal stages, so are not exposed to environmental alterations occurring in the aging brain. Moreover, important transcriptional differences exist between neonatal and adult astrocytes (Cahoy et al., 2008). To overcome this problem, other groups have isolated astrocytes directly from aged rats (Jiang and Cadenas, 2014). Despite presenting an advantage over the "aging in a dish" models, one important constraint of these *in vitro* studies is that astrocytes are grown in serum-containing media, which triggers astrocyte reactivity (Foo et al., 2012). Using these aging *in vitro* cultures, it has been shown that cortical astrocytes isolated from aged (18 month-old) rats illustrated an age-dependent increase in mitochondrial oxidative metabolism compared to astrocytes isolated from adult rats (7-13 month-old) (Jiang and Cadenas, 2014). Furthermore, aged astrocytes displayed an enhanced response (such as increased induced nitric oxide synthetase (iNOS), p38 and pJNK pathways) to inflammatory cytokines. Interestingly, an astrocyte aging transcriptome study showed that 2 -year-old mice treated with lipopolysaccharide (LPS) to induce inflammation, yielded a significantly increased expression of markers for astrocyte reactivity compared to young mice (Clarke et al., 2018). This further corroborates an altered inflammatory response of astrocytes with aging. Advances in isolation and culture methods for obtaining physiologically aged astrocytes to use in *in vitro* studies is essential for better understanding the contributions of astrocytes to brain aging.

2.5.2. Ex vivo approaches

Another method to characterize astrocyte physiology and astrocyte-neuronal communication in the aging brain is the use of *ex vivo* brain slices. To this end, Lalo et al., isolated somatosensory cortex slices from aged (18 to 21 months old) and adult (6-month-old) mice and measured astrocyte membrane currents using electrophysiology recordings. They reported a decrease in the size of P2X, NMDA, and AMPA receptor-mediated currents in aged astrocytes in response to electric stimulation of cortical axons from layer IV-VI neurons (Lalo et al., 2011). Similarly, Gómez-Gonzalo *et al.* showed an impaired ability of aged astrocytes to release glutamate upon mGluR activation in mouse hippocampal and cortical slices (Gómez-Gonzalo et al., 2017). The first study also reported a decrease in astrocyte somatic Ca^{2+} transients upon neuronal stimulation in aged mice (Lalo et al., 2011). This observation was further confirmed in a later publication from the same group (Lalo et al., 2018). In contrast, Gomez-Gonzalo *et al.* demonstrated no changes in somatic astrocyte intracellular calcium signals, either spontaneous or in response to different receptor agonists or electric stimulation (Gómez-Gonzalo et al., 2017). The different outcomes from these studies might be explained by the different readouts used to quantify astrocytes Ca^{2+} excitability: while Lalo et al. measured Ca^{2+} peak amplitude and frequency, Gomez-Gonzalo quantified Ca^{2+} spike probability. Additionally, the probes used to measure intracellular Ca^{2+} were also different in each study (Fura-2 versus Fluo-4).

To further investigate the role of astrocyte Ca^{2+} signalling in the aged brain, Guerra-Gomes and colleagues used a IP3R2 knock-out (KO) model, in which Ca^{2+} release from internal stores in astrocytes is decreased (Guerra-Gomes et al., 2018). Remarkably, aged (18-month-old) IP3R2 KO mice did not display the age-associated memory impairments shown in the wild type mice, suggesting that astrocyte Ca^{2+} signalling may play an active role in mediating synaptic dysfunction and memory defects in aged mice.

In summary, despite the technical limitations to characterize how brain aging affects astrocyte function, most of these studies demonstrate that this function is compromised with aging: aged astrocytes show impaired neuronal-astrocyte communication, decreased support to neurons and synapses, and illustrate a pro-inflammatory phenotype. Further supporting an impaired role of astrocytes with aging, one publication reported altered astrocyte- specific responses to acute traumatic brain injury in aged mice (Early et al., 2020). Again, it is important to stress that alterations in astrocyte function with age might be different depending on the brain region, species or sex analysed.

3. Microglial alterations with aging

3.1. Properties of microglia

Microglia are the resident immune cells of the brain, constituting 10% of brain cells. (Crotti and Ransohoff, 2016). They originate from myeloid progenitors in the yolk sac and play important physiological roles in health and disease. "Resting" microglia display a ramified morphology with motile processes. They are constantly surveying the CNS microenvironment and interacting with neuronal cell bodies, synapses, astrocytes, and blood vessels (Li and Barres, 2018; Szalay et al., 2016). Microglia are known for their ability to respond to brain disease and injury (Davalos et al., 2005; Ransohoff and Perry, 2009). They can rapidly react and migrate towards a site of injury such as neuronal death, pathogen infection, or blood brain barrier leakage (Nimmerjahn et al., 2005). Once activated, microglia change their morphology, shortening cytoplasmic processes and enlarging the soma. These morphological changes are accompanied by functional alterations: they acquire a macrophage-like structure, efficiently clearing dying cells and cellular debris (Kettenmann et al., 2011; Napoli and Neumann, 2009). Microglia can also release cytokines and chemokines to stimulate other cells (Ransohoff and Perry, 2009).

Microglia are not only important for their immune roles, but also play key homeostatic functions in the healthy brain. During development they can respond to changes in neuronal activity, regulating synapse formation, maturation, and elimination (Schafer and Stevens, 2015; Stevens et al., 2007). In the adult brain microglia can modulate hippocampal neurogenesis (Gemma and Bachstetter, 2013), myelination, and synaptic plasticity, thus affecting behaviour (Hagemeyer et al., 2017; Zhou et al., 2019). These glial cells are being increasingly implicated in age-related neurodegenerative diseases such as Parkinson's and Alzheimer's disease (McQuade and Blurton-Jones, 2019). Thus, understanding how microglial cells change with aging is becoming a key question to clarify their contribution to neurodegeneration. In this section we will discuss what happens to microglia during physiological aging: Do microglia respond to brain aging the same way they respond to injury? Is aging perceived as a pathological insult by these resident immune cells?

3.2. Does microglia morphology change with aging?

In response to activation, microglia change their morphology, acquiring a macrophage-like round shape, usually accompanied by enhanced immune functions (Crotti and Ransohoff, 2016). Therefore, characterising how microglia change their morphology with aging is important to understand how their function might be affected.

An early electron microscopy study characterizing alterations in glia in aged rats reported an increase in glial cell size and large accumulations of membrane-bound inclusion materials, closely resembling lipofuscin deposits (Vaughan and Peters, 1974). Notably, microglia alterations were more dramatic than the ones observed in astrocytes or oligodendrocytes. This observation was corroborated in later studies using aged mice and monkeys (Peters et al., 1991; Samorajski, 1976). A more recent publication analyzed microglia morphology and dynamics *in vivo* using 2-photon microscopy in mouse neocortex. They showed

similar morphological alterations occurring with age, including increased soma volume and shortening of processes in old mice (24-month-old) compared to young ones (3 and 12 month-old) (Hefendehl et al., 2014).

Some of these morphological alterations are similar to the ones shown in activated microglia (Conde and Streit, 2006). However, there are important differences between activated microglia and aged microglia: activated microglia appear locally in response to a specific injury, and they are enlarged due to an acute phagocytosis of tissue debris. In contrast, aged microglia appear relatively uniformly across the CNS and are enlarged due to accumulation of non-degraded waste material throughout their entire lives as scavengers (Streit and Xue, 2010).

Interestingly, with aging, human brains show specific microglial alterations that are not present in mice (Streit, 2006). These changes include beaded and fragmented processes accompanied by an abnormal/disrupted cytoplasmic structure. These cells were termed dystrophic microglia, and are thought to reflect microglia degeneration (Streit et al., 2004). Notably, in mice, aging is not sufficient to induce microglial dystrophy, and even microglia in the senescence accelerated mouse model (SAMP-10) do not show signs of cytoplasm fragmentation (Kawamata et al., 1998). This human-specific presence of dystrophic microglia in the aged brain could be explained by the difference in lifespans between mouse and humans (Streit et al., 2014). It is also important to remember that most lab rodents are kept in pathogen-free facilities and unchanged environments, conditions very different from a long-lived human being.

3.3. Do microglia numbers alter with aging?

In response to CNS stressors such as nerve injury microglia can proliferate (Salter and Stevens, 2017). Whether numbers of microglial cells are altered with aging is still not clear, as studies addressing this question have shown conflicting results. Early electron microscopy studies identified different type of glia cells using morphological features and suggested a robust increase (45-65%) in cortical microglia with aging in rats (Vaughan and Peters, 1974) and monkeys (Peters et al., 1991). However, a more recent electron microscopy study in human neocortex reported no differences in microglia number in aged individuals. These studies used only morphological parameters for cell identification, and as the authors acknowledge in the paper, “distribution of features may change when specific stains are applied”(Fabricius et al., 2013).

To overcome this limitation other groups have analysed cell number changes with aging using antibodies against specific microglia markers such as ionized calcium binding adaptor molecule 1 (Iba1). However, the outcomes from these studies are not always in agreement. While some authors reported an increased number of cortical microglia in aged monkeys (Robillard et al., 2016) and mice (Tremblay et al., 2012), others observed no differences in Iba1+ microglia numbers in the hippocampus from old rats (26 month-old) compared to adult ones (12 month-old) (VanGuilder et al., 2011). Another study even reported a decreased number of microglia and signs of microglia degeneration in the substantia nigra and striatum in 18 and 24 month-old mice (Sharaf et al., 2013).

The variability of these results could be due to the different brain regions characterized (cortex versus hippocampus or striatum) in each study. This variability highlights the possibility that microglia, similarly to astrocytes, change differently with aging in a region-specific manner as other studies have suggested (Grabert et al., 2016). A more systematic spatial characterization of microglia changes with aging would be necessary to address this question.

3.4. Does the microglia transcriptome change with aging?

One approach to understand alterations of microglia with aging is differential transcriptional analysis. By characterizing changes in gene expression associated with aging, we can identify the different pathways affected and the functions that may be compromised in the aged microglia.

An early microarray analysis revealed a transcriptional signature of activated microglia in aged mice (20-24 month-old) compared to young ones (3-6 months). In addition, the inflammatory response upon LPS stimulation was exacerbated with aging (Godbout et al., 2005). Interestingly, aged astrocytes also showed an increased reactive response to LPS stimulation (Clarke et al., 2018). This suggests that both astrocytes and microglia share increased susceptibility to neuroinflammation in the aging brain. Another study performed RNA sequencing of microglia isolated from adult (5-month-old) and aged (24-month-old) mice. Aged microglia showed a downregulation of genes involved in endogenous ligand recognition (i.e.: . cell debris), and an upregulation of genes important for microbe recognition, host defense, and neuroprotection (Hickman et al., 2013). A more recent report isolated aging microglia containing high levels of lipid-droplets (LD-high) from 20-month-old mice hippocampi, and studied their transcriptional profile compared to microglia with low levels of lipid droplets (LD-low). Consistently, these lipid-droplet-accumulating microglia show changes in gene expression associated with defects in phagocytosis, produce higher levels of reactive oxygen species, and secrete proinflammatory cytokines (Marschallinger et al., 2020). To identify different immune cell populations present in the aged mouse brain, Mrdjen et al. recently performed high dimensional single-cell mass cytometry (Mrdjen et al., 2018). Using this approach, authors found a phenotypic signature in a subset of microglia in geriatric mice (18 months) compared to young (2 months). Interestingly, a mouse model for Alzheimer’s disease showed a similar phenotypic signature as present in aged mice.

These analyses were performed on microglia isolated from the whole brain or hippocampus. Given that aging affects brain regions differently, Grabert *et al.* characterized the mouse aging microglia transcriptome in different brain regions to understand whether microglia show region-specific transcriptional changes with aging, similar to those observed in astrocytes. Overall, cerebellar microglia showed an upregulation of genes involved in immune response, whereas hippocampal microglia lost their region-specific distinctive gene expression, converging with cortical and striatum samples. Genes downregulated in aged hippocampal microglia were involved in phagocytosis, cytoskeletal reorganization, cell adhesion, and motility, supporting an idea of a disengagement of aged microglia with the environment. These regional differences “could underlie region-specific sensitivities to microglial dysregulation and involvement in age-related neurodegeneration” (Grabert et al., 2016). Interestingly, another transcriptional study addressed the differences in hippocampal microglial aging between male and female mice, showing an enhanced inflammatory response in microglia from old females compared to old males (Mangold et al., 2017). These sex-specific alterations in hippocampal microglia with aging could also contribute to the differential vulnerability to develop age-dependent neurodegenerative disease in men and women.

Considering the human-specific changes in microglia morphology observed in aging, it is important to analyze whether transcriptional changes are also different between aged mouse and humans. To this end, Galatro *et al.* performed a transcriptome analysis of human microglia purified from parietal cortex of 39 subjects. With aging, human microglia showed alterations in genes important for cell adhesion, axonal guidance and actin disassembly. Surprisingly, these changes showed little overlap with previously generated gene expression profiles of aging mouse cortical microglia (Galatro et al., 2017). Other microglia transcriptional studies using human brain autopsies from aged individuals also reported the presence of age-specific genes involved in DNA damage, telomere maintenance and phagocytosis, that were not present in young human microglia from other databases (Olah et al., 2018). Furthermore, the same publication showed an increased expression of susceptibility genes for sporadic Alzheimer’s disease and

multiple sclerosis in human aged microglia.

3.5. Does microglia function change in the aging brain?

With aging, microglia change morphology and become dystrophic, deramified and spheroid, but what are the consequences of these age-associated alterations on microglial function?

3.5.1. In vitro approaches

One approach that researchers have taken to characterize functional changes of microglia with aging is the use of *in vitro* microglia cell cultures from aged mice. However, similarly to what happens with astrocytes, keeping "healthy" microglia *in culture* have been hindered: microglia generally lose their ramified processes and acquired features of activated microglia (Bohlen et al., 2017). Thus, outcomes from studies using these *in vitro* approaches must be taken with caution.

One study isolated whole-brain microglia from aged (14-16 month-old) and young (1-2 months) mice using a densitiy centrifugation protocol optimized to clear up cellular debris. Results suggest that aged microglia increase their pro-inflammatory function, secreting higher levels of the pro-inflammatory cytokines IL-6 and TNF-α and showing an enhanced inflammatory response upon LPS stimulation (Njie et al., 2012). Additionally, the ability to internalize amyloid-beta peptide is reduced in microglia isolated from aged mice.

3.5.2. In vivo approaches

A different approach to characterize functional changes in aged microglia is the use of *in vivo* rodent models at advanced ages (> 18 month-old).

First, to study whether microglia response to damage are affected with aging, Conde et al. characterized microglia proliferation after facial nerve axotomy. Authors reported significantly increased proliferation of microglia in old (30 months) rats compared to young ones (3 months) (Conde and Streit, 2006). Surprisingly, aged microglia, despite displaying an enhanced proliferative response upon stimulation, demonstrate impaired motility (Damani et al., 2011; Hefendehl et al., 2014). Another study compared dynamics of retinal microglia from young (3 month-old) and aged (18-24 month-old) mice *in situ* using live imaging reported an altered response of aged microglia in response to injury signals (delivered by extracellular ATP or laser-induced focal injury): while young microglia became ramified and increased their motility, aged microglia yielded slower and less dynamic responses (Damani et al., 2011). A different group showed very similar results using 2-photon microscopy to analyse microglia dynamics in response to laser lesion in 24-month-old mouse neocortex (Hefendehl et al., 2014). In spite of the differences existing between retinal and cortical microglia, (Li et al., 2019) it is very interesting that both groups reached the same conclusions of impaired microglia dynamics in response to injury.

Similarly to astrocytes, microglia are able to coordinate and respond to activating stimuli using intracellular Ca^{2+} signalling (Färber and Kettenmann, 2006). To characterize whether these signals are affected with aging, a recent publication used 2-photon microscopy to monitor microglia Ca^{2+} transients *in vivo* in the aged (18-21 month-old) mouse cortex (Del Moral et al., 2019). Authors observed decreased frequency and amplitude of microglia spontaneous Ca^{2+} transients in aged mice compared to adult (9-11 month-old). Additionally, the same study reported that in aged mice, cortical microglial processes moved faster but in a more disorganized manner in response to an ATP source, compared to young adult mice.

3.5.3. Microglia replacement approaches

These functional studies point towards an altered function of microglia with aging. But, are these changes in microglia a compensatory response to protect against brain aging, or are they detrimental, contributing to age-dependent alterations? To tackle this question, different groups have performed replacement studies to characterize the effect of aged microglia depletion on brain function, using a colony-stimulating factor 1 receptor (CSF1R) inhibitor, which causes a rapid depletion of microglia (Elmore et al., 2014). Elmore et al. reported that 28 days after microglia depletion and natural repopulation, 24-month-old mice show improved spatial learning, and increased hippocampal dendritic spines and neurogenesis (Elmore et al., 2018). In contrast, O'Neil *et al.* using a similar method for microglia depletion, illustrated that replenished microglia cells, despite showing no age-associated morphological alterations, still adopt an enhanced pro-inflammatory profile in the aged brain (O'Neil et al., 2018). The authors speculated that the microenvironment from the aged brain primes microglia to adopt a pro-inflammatory profile.

Taken together, these data demonstrate that with aging microglia undergo changes in morphology, accumulating non-degraded inclusion materials and cytoplasmic disruptions. These changes are accompanied by alterations in their physiological function: aged microglia display an enhanced pro-inflammatory response, impaired motility, and a compromised immune response. All of these alterations may contribute to the altered neuronal function and the cognitive deficits that develop with age.

4. Oligodendrocytes in the aging brain

4.1. Properties of oligodendrocytes

Oligodendrocyte precursor cells (OPCs) give rise to oligodendrocytes, the myelin producing cells in the developing and adult brain. Importantly, OPCs persist in the adult brain, awaiting cues to differentiate into new oligodendrocytes in response to learning, injury, disease, or aging (Bergles and Richardson, 2016). The primary role of oligodendrocytes is to myelinate neuronal axons, thus providing electrical insulation that increases conduction velocity and forms an axon-myelin unit. Myelin sheaths are formed by multi-layered stacks of electron-dense, condensed cytoplasmic surfaces of oligodendrocytes that prevent current leakage (Stadelmann et al., 2019). Myelinated segments between the nodes of Ranvier are called the internodes. The edges of each myelin segment, called the paranodal domains, are contacted by astrocyte processes thus creating an extensive network of glial cells. Notably, myelin produced later in adulthood is characterized by thinner sheaths and shorter internodes (Blakemore and Murray, 1981; Lasiene et al., 2009; Young et al., 2013). Remyelination after degeneration, such as in multiple sclerosis or injury, also results in shorter internodes, which can lead to a decrease in the conduction rates along the nerve fibers. Oligodendrocytes perform important functions besides myelinating axons. Signals from oligodendrocytes are neurotrophic and neuroprotective, facilitating axonal function as well as general health and survival of neurons (McTigue and Tripathi, 2008). Interestingly, dysfunctional oligodendrocytes and/or myelin can be responsible for axonal dysfunction and degeneration without apparent demyelination (McTigue and Tripathi, 2008).

As highly metabolically active cells playing critical roles in action potential propagation and neuronal support, oligodendrocyte homeostasis is likely vulnerable to changes in the brain environment. Accordingly, these cells are frequently implicated in CNS injury and disease. Structural and functional alterations to oligodendrocytes and OPCs are observed in multiple sclerosis, Alzheimer's disease, Parkinson's disease, ischemia, and spinal cord injury (Ettle et al., 2016). In this section, we will discuss the primary changes to oligodendrocytes across aging. Are oligodendrocytes more susceptible to dysfunction and degeneration in the aging brain, thus decreasing their support for neuronal function?

4.2. Does oligodendrocyte morphology and myelin structure change with aging?

Some of the earliest studies investigating aging-induced changes to oligodendrocytes focused on morphological alterations to these cells, observed by electron microscopy of young (4-10 years old), middle aged (12-19 years old) and old (over 25 years of age) rhesus macaque neural tissue. In particular, analyses of oligodendrocytes in the frontal and primary visual cortices of rhesus macaques demonstrated that aged cells contain dense inclusions in the cytoplasm of cell bodies and swellings along their processes (P, 1996; Peters et al., 1991). A follow-up study from the same group found similar changes in the prefrontal cortex and corpus callosum of aged (over 25 years of age) male and female rhesus monkeys (Peters and Sethares, 2004). Reviewing the evidence, Peters hypothesized that the components of these dense inclusions were derived from myelin sheaths that have been renewed (Peters, 2009). These early indications of aging-related morphological alterations to oligodendrocytes and myelination ignited more focused research into the role of these specialized cells in the aging brain.

Magnetic resonance imaging (MRI) has demonstrated that subcortical white matter volume decreases with age in healthy humans and monkeys (Bartzokis et al., 2001; Guttmann et al., 1998; Wisco et al., 2008). Researchers proposed that these changes in white matter volume could be due to alterations in the myelination of axons in cortical and subcortical regions. Modifications to the structure of myelin sheaths can induce significant changes to neuronal function (reviewed in (Peters and Sethares, 2002)). These effects can lead to downstream cognitive decline and other behavioral deficits. Notably, changes to myelination can appear prior to cognitive deficits or disease diagnosis (e.g. Alzheimer's disease) (Bartzokis, 2011). Using electron microscopy, several studies investigating myelin sheaths across different ages in rhesus monkeys found alterations in the myelin structure in the prefrontal cortex, primary visual cortex, and corpus callosum (Peters et al., 2000; Peters and Sethares, 2002). The most commonly observed change was splitting of the dense lines of myelin sheaths that accommodated pockets of dense cytoplasm from the oligodendrocyte. These morphological changes to the myelin in old monkeys is termed dense degeneration. Furthermore, these studies illustrated a relationship between age-related changes to the number of oligodendrocytes and the frequency of altered sheaths, paranodes, and internodes. Peters and colleagues concluded that age induces degeneration of myelin and/or oligodendrocytes, which leads to production of new oligodendrocytes that remyelinate axons. Myelination by new oligodendrocytes produce shorter and more frequent internodes. In agreement, Tremblay and colleagues also found ballooning and redundant myelin sheaths in the aged (20 month) mouse cortex, which could influence action potential conduction velocity in these neurons (Tremblay et al., 2012). The authors hypothesize that these changes are due to oligodendrocytes' attempts to replace degraded or dysfunctional myelin. However, direct evidence for the differentiation of new oligodendrocytes that then remyelinate axons was not shown.

Taken together, these studies indicate that myelin experiences significant morphological remodelling in the aging brain, possibly contributing to the observed aging-related deficits in neuronal and cognitive function.

4.3. Does aging alter myelin plasticity and remodelling?

Much like myelination is important for neuronal function, neuronal activity and plasticity also induce changes to myelin (Fields, 2014; Stevens et al., 2002; Wake et al., 2011). Of note, enhanced neuronal activity through optogenetic stimulation results in increased oligodendrogenesis and myelination (Gibson and Lin, 2014). In these experiments, stimulation of the premotor cortex of mice promoted myelination in deeper cortical layers and subcortical white matter and improved motor function. Although this study did not investigate the effects of aging, it is in line with the notion that neuronal activity plays an important and substantial role in oligodendrogenesis and myelination.

To address how aging-related changes to neuronal activity influence oligodendrocytes and myelination, Tremblay and colleagues compared structural changes to neurons and glial cells in the context of aging and age-related sensory loss (Tremblay et al., 2012). Researchers used two mouse strains that individually exhibit either age-related visual (CBA) or hearing (C57) loss to investigate changes driven by aging or a combination of aging and sensory loss. Immunocytochemical electron microscopy of primary visual and auditory cortices of young (3 month) and aged (20 month) mice revealed ultrastructural changes to oligodendrocytes and axonal myelination. Specifically, the authors observed ballooning of myelin sheaths and redundant sheaths in cortical regions of aged CBA and C57 mice, especially in the regions associated with sensory loss (primary visual cortex of CBA mice and primary auditory cortex of C57 mice). These morphological changes are evidence of demyelination/remyelination which is more apparent in cortical regions associated with sensory loss. Tremblay and colleagues concluded that myelin remodelling and axonal dysfunction could be exacerbated by sensory loss in normal aging. These experiments did not directly investigate activity-dependent myelination or myelin remodelling in the aging brain.

Recent advances allow for analyses of myelin dynamics with high spatial and temporal resolution in mouse brains (Hill et al., 2018). *In vivo* recordings of structural myelin plasticity in adult and aged mice (2 years old) illustrated that new internodes were being added to partially myelinated axons into adulthood as new oligodendrocytes were generated. However, these data indicated that plasticity was reversed at advanced ages, with little formation of new internodes and increased myelin degeneration. Of note, this study demonstrated that myelin debris accumulated in microglia in the aged brain. In the aging brain, microglia play an important role in myelin turnover and clearance of pieces released by aging myelin sheaths (Safaiyan et al., 2016). These pieces of myelin result in insoluble, lysosomal inclusions found in microglia, which may lead to immune responses frequently observed in aging (Marschallinger et al., 2020).

Similarly, Cantuti-Castelvetri and colleagues found that myelin regeneration capacity is reduced in aged mice (Cantuti-Castelvetri et al., 2018). After inducing focal demyelinating lesions, the authors found that lesion area and volume were larger in aged (12 month) mice compared to young (3 month) mice 2 weeks after demyelination. Inflammatory responses also lasted longer in the aged mice, and aged microglia accumulated more myelin debris. The authors found that increasing cholesterol transport in microglia was able to restore remyelination in old mice. Although this study uses 12-month mice and not older, it provides evidence for impaired myelin regeneration in aging.

In future, studies combining analyses of myelin dynamics with OPC proliferation and differentiation *in vivo* will be beneficial for determining how aging-related changes to neuronal activity influence oligodendrogenesis and myelination.

4.4. Do oligodendrocyte numbers change in the aging brain?

As the myelinating cells in the brain, changes to numbers of oligodendrocytes result in significant consequences to cognitive and neural functions. Evidence points to both an increase and decrease in the number of oligodendrocytes across aging, making this topic an open matter of discussion. A study of brain tissue from human females found fewer oligodendrocytes in subjects over 90 years old (Fabricius et al., 2013). Of note, cells were identified using morphological features, and the authors acknowledge that the lack of immunohistochemical analyses and reliance on only morphological criteria could lead to incorrect categorization of cells. In agreement with this study, immunohistochemical analyses of human post-mortem brain tissue from

males and females (18 to 93 years old) found an age-related decrease of oligodendrocytes in the frontal cortex, but not in other cortical regions (Pelvig et al., 2008). Another assessment of oligodendrocytes via immunohistochemical analysis of human post-mortem brain tissue also found a decrease in oligodendrocytes in the frontal cortex of aged (above 75 years of age) individuals (Soreq et al., 2017). In this study, analysis of gene expression datasets from human brain tissue also illustrated a downregulation of genes involved in oligodendrocyte differentiation, including precursors, genes encoding myelin components and myelin regulatory genes, further implying changes in myelination capacity with aging (Soreq et al., 2017). *In vivo* imaging of oligodendrocytes across adulthood and into old age found that oligodendrocyte density decreases in aged mice (2 years old) (Hill et al., 2018).

Conversely, other studies have suggested that oligodendrocyte numbers increase in the aging brain. In the primary visual cortex of aging male and female rhesus monkeys, Peters and colleagues found that the oldest monkeys with the greatest cognitive deficits had the most oligodendrocytes and smaller internodes in the myelin sheaths (Peters, 2009; Peters and Sethares, 2004). They concluded that more oligodendrocytes were required to remyelinate the degraded sheaths that developed in the aging cortex. An increased number of oligodendrocytes was further observed in the cortices of old (20 months) male and female mice using morphological categorization and electron microscopy (Tremblay et al., 2012). In these experiments, an increase in the number of oligodendrocytes was observed in the respective primary sensory cortices of two inbred mouse strains that separately exhibit age-related loss of audition and vision.

Whether oligodendrocyte numbers increase or decrease in the aging brain still requires further investigation. It is possible that variability among oligodendrocyte numbers and susceptibility of different brain regions to aging-induced alterations may be responsible for some of these seemingly contradictory results. Further, these contradictory results may be due to methodological differences among the research groups. For example, while some studies only include female subjects (Fabricius et al., 2013), others used both males and females and combined sexes in the analysis (Peters, 2009; Soreq et al., 2017; Tremblay et al., 2012).

4.5. Does OPC proliferation and differentiation change with aging?

One way to investigate alterations to oligodendrocyte populations is to determine whether OPC proliferation and differentiation is being altered in the aging brain. OPCs generate new myelinating oligodendrocytes in the adult mouse brain (Rivers et al., 2008). Therefore, if aging influences the rate by which OPCs produce mature oligodendrocytes, this may explain the observed alterations in myelin in the aging brain. A study of dividing cells in the spinal cord of mice across age indeed supports the hypothesis that glial cell proliferation increases in aged animals, possibly explaining the increased number of oligodendrocytes in some of the studies described above (Lasiene et al., 2009). In these experiments, dividing cells were labelled using BrdU and characterized by staining with glial cell markers. Cell proliferation was highest in white matter at 21 months and the majority were NG2 and Olig2 positive, a marker of early fate restriction to the oligodendrocyte lineage. A smaller percentage were also CC1 positive, a marker that indicates more mature oligodendrocytes. This increase in proliferation of glial precursors and subsequent increase in oligodendrocytes in the aging spinal cord may be a compensation mechanism due to aging-related demyelination and dysfunction of existing oligodendrocytes.

Conversely, a study published in 2019 concluded that OPCs are less abundant in the aged mouse brain (Ximerakis et al., 2019). Single cell transcriptomic profiling of mouse brains revealed a decrease in the number of OPCs in samples from 21-22 month old mice (Ximerakis et al., 2019). However, isolation procedures for single-cell analyses may introduce biases towards specific cell populations, so may not be a reliable method for measuring cell numbers. Nonetheless further differential gene expression and pathway analyses illustrated that genes encoding ribosomal subunits were downregulated in OPCs from aged mice, while aged neurons experienced an upregulation of these genes. These bidirectional effects of aging strongly support the importance of studying cell-specific alterations in the aging brain. The authors postulate that aging differentially affects cells with different metabolic demands. For example, translational efficiency and protein synthesis may be differentially affected in OPCs when compared to neurons, which results in differing compensatory mechanisms observed by transcriptomic analyses.

This study also supports the hypothesis that aging induces a slowing down of OPC differentiation which in turn results in a decrease in mature oligodendrocytes in the aged brain. However, it is unclear whether cell death is greater in these aged-OPCs or if they are less able to differentiate into mature oligodendrocytes. Also, as discussed in the previous section, studies have found both increases and decreases in oligodendrocyte number. These differing results in both oligodendrocyte and OPC population sizes may be partially explained by the differences in tissue processing and analysis methods employed in these experiments. These studies each primarily utilized a single analysis method: either single-cell RNA-sequencing or immunohistochemistry. It is possible that by combing methodologies, we can better address the aging-induced changes to these specialized cell populations.

5. General conclusions

Astrocytes, microglia, oligodendrocytes and OPCs shape nervous system and neural circuit development and further influence synapse function, homeostasis, and plasticity in adulthood. In response to aging, glial cells experience morphological and functional alterations that reduce their neuroprotective roles: microglia display an enhanced inflammatory response, astrocytes decrease synaptic support, and oligodendrocytes modify their myelination capacities (Figure 1). These alterations result in decreased neuronal support and increased neuronal vulnerability, priming the brain to age-associated disorders including cognitive decline, vascular alterations and neurodegenerative diseases.

Glial cells do not act in isolation but interact with neurons and other glial cells, creating an extensive network of interconnected cells. Aging-related alterations to the functions of one cell type can escalate the pathological cascade occurring with aging. For example, myelin pieces released upon myelin fragmentation with aging are taken up by microglia, contributing to the formation of lipofuscin-like lysosomal inclusions. This accumulation of insoluble material might compromise microglia function (Safaiyan et al., 2016). In turn, microglia can signal to astrocytes to induce reactive changes (Liddelow et al., 2017). Thus, initial glial dysfunction might be the first step triggering the pathological changes in aging, predisposing the brain to neurodegenerative disorders. Supporting this hypothesis, Bussian and colleagues investigated the role of senescent glial cells in a mouse model of tau-dependent neurodegenerative disease (MAPTP301SPS19) (Bussian et al., 2018). In this study, the authors show the presence of astrocytes and microglia expressing the senescence marker p16INK4A. Clearing these cells decreases gliosis and neuronal death, and preserves cognitive function in these mice. However, it is unclear which specific glial cell types are responsible for the prevention of neurodegeneration and cognitive deficits in these experiments.

In this review, we discuss aging-associated changes to glial cells that are region- and species-specific. This regional specificity of glial changes in response to aging may explain why certain brain regions are more susceptible to synaptic and neuronal dysfunction in aging and neurodegenerative diseases. Hippocampal astrocytes and microglia appear more susceptible to aging-induced morphological and transcriptomic changes (Cerbai et al., 2012; Grabert et al., 2016; Soreq et al., 2017). Loss of specific synaptic connections in the hippocampus in aged animals has been shown, which correlates with the glial

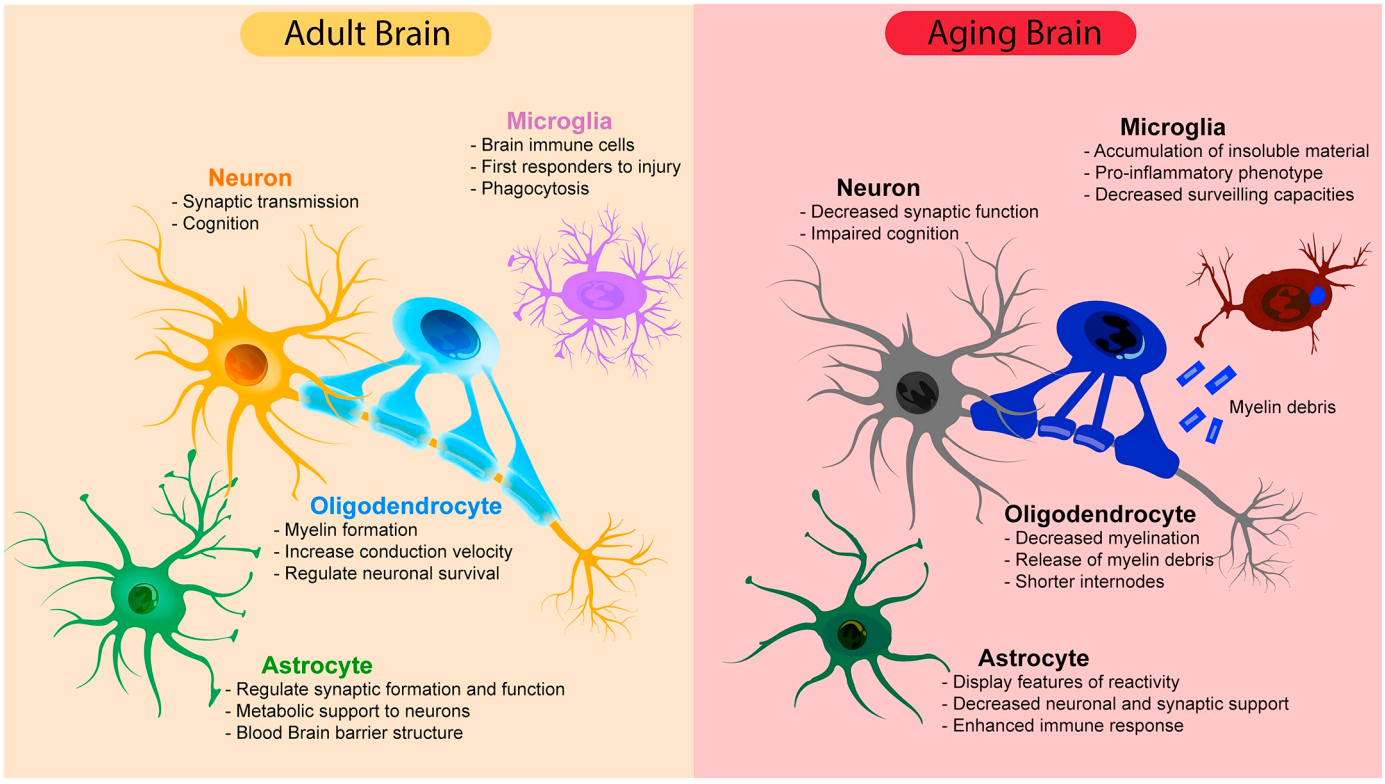

Fig. 1. Schematic showing major alterations to glial cell function between the adult and aging brain.

changes observed in this brain region (Burke and Barnes, 2010). Many studies have also evaluated synapse loss and glial changes in the frontal cortex. The frontal cortex seems to experience synapse loss and synaptic deficits associated with aging, as well as alterations in the transcriptome and properties of glial cells (Brennan et al., 2009; Dumitriu et al., 2010; Mathys et al., 2019; Tachibana et al., 2016). These studies point to a relationship between aging-associated synapse loss and dysfunction and glial changes in susceptible regions. These regions are implicated in cognitive functions, such as memory formation and executive functions, that are frequently associated with age-related decline. However, these studies only show a correlation, and more systematic research is needed to elucidate how glial cells are changing in different brain regions and how these changes are linked to region-specific synapse dysfunction and loss.

Interestingly, different mouse transcriptome analyses have shown that in the cerebellum, a brain region not linked to age-related cognitive decline, microglia and astrocytes experience the largest changes in gene expression with aging compared to other brain regions (Boisvert et al., 2018; Grabert et al., 2016). Thus, it is also possible that age-related changes to glial cells may be protective. Further research is needed to elucidate which aging-associated alterations to glial cells are neurotoxic or neuroprotective.

We further illustrated the limitations of many aging studies due to differences in methodologies, such as which regions and species were studied. Nonetheless, the development of -omics techniques, such as single-cell transcriptomics, metabolomics, and proteomics, and standardization and expansion of bioinformatics tools increase the feasibility of studying both glia and neurons across regions in the aging brain (De Monasterio-Schrader et al., 2012; Jha et al., 2013; Mathys et al., 2019). Advances in imaging techniques, such as *in vivo* imaging and production and validation of glial tracers and markers, further allow for greater spatial and temporal resolution. With these tools, researchers can better evaluate how glial cells are changing and interacting with each other to modulate and respond to neural activity and how alterations in these functions and relationships with aging can result in neural circuit dysfunctions that lead to cognitive deficits. Further research into early alterations to glial cells in the aging brain may identify novel therapeutic targets for the treatment of aging-associated disorders

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