

# Adult Astrogenesis and the Etiology of Cortical Neurodegeneration

## Supplementary Issue: Molecular and Cellular Mechanisms of Neurodegeneration

Tal C. Mohn and Andrew O. Koob

Biology Department, University of Wisconsin—River Falls, River Falls, Wisconsin, USA.

**ABSTRACT:** As more evidence points to a clear role for astrocytes in synaptic processing, synaptogenesis and cognition, continuing research on astrocytic function could lead to strategies for neurodegenerative disease prevention. Reactive astrogliosis results in astrocyte proliferation early in injury and disease states and is considered neuroprotective, indicating a role for astrocytes in disease etiology. This review describes the different types of human cortical astrocytes and the current evidence regarding adult cortical astrogenesis in injury and degenerative disease. A role for disrupted astrogenesis as a cause of cortical degeneration, with a focus on the tauopathies and synucleinopathies, will also be considered.

**KEYWORDS:** astrocyte, gliogenesis, neurogenesis, gliosis, Alzheimer's disease, dementia with Lewy bodies

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**CORRESPONDENCE:** andrew.koob@uwrf.edu

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## Introduction

Mature astrocytes in the cerebral cortex are capable of local proliferation under certain conditions, with indications that their progeny may resume normal physiological function over time.<sup>1,2</sup> It is known that injury and degeneration can cause reactive astrogliosis and subsequent astrocyte proliferation, although the exact molecular stimulus of proliferation is currently unknown.<sup>3</sup> The process whereby reactive astrocytes proliferate was originally considered detrimental, as after injury or stroke, glial scar formation inhibited axonal regrowth in the area.<sup>4</sup> However, reactive astrogliosis and astrocyte proliferation are now understood to be neuroprotective, provide factors to promote cell survival, and in severe lesions in injury and degeneration seal off an area of robust necrosis.<sup>1,5,6</sup>

One of the hallmarks of neurodegenerative disease is synapse loss,<sup>7</sup> which occurs early in disease and has long been associated with cognitive decline in cortical dementias, such as Alzheimer's disease (AD), Parkinson's disease dementia, and dementia with Lewy bodies (DLBs).<sup>7–9</sup> Protoplasmic astrocytes in the cortical gray matter are closely associated with synapses and synapse monitoring.<sup>10–12</sup> Recent evidence points to astrocytes in the cortex as contributors to synaptogenesis in response to neuronal communication<sup>13,14</sup> and responsible for general control of synapse number.<sup>15</sup>

Astrocytes are responsible for a variety of homeostatic functions that can lead to neurodegeneration if unchecked. Astrocytes supply neurons with glutathione precursors and

protect neurons from cell death as a result of reactive oxygen species production.<sup>16</sup> Clearance of toxins from the parenchyma occurs through astrocytes via the glymphatic system.<sup>17–19</sup> Astrocytes also remove glutamate through glutamate transporters to avoid excitotoxicity and neuronal cell death.<sup>20</sup> It is no longer disputed that astrocytes express the same transmitters and receptors as neurons, are active contributors in central nervous system communication,<sup>21</sup> and maintain ionic and osmotic homeostasis in the brain.<sup>22</sup>

Astrocytes monitor and regulate cerebral blood flow in the cortex.<sup>23,24</sup> This is particularly significant as local astrogenesis occurs in the cortex perivascularly,<sup>2</sup> and vascular defects are commonly associated with cortical dementia.<sup>25</sup> Astrocytes also remove and clear amyloid- $\beta$  from the extracellular space before the accumulation of the protein into amyloid plaques, the pathological hallmark of AD.<sup>17</sup>

Indeed, because of the ubiquitous number of functions involved, there is increasing evidence that many neurodegenerative diseases are astrocytic in nature,<sup>26–29</sup> and it is becoming clear that astrocytes are an important avenue for the treatment and prevention of neurodegenerative disease.<sup>30,31</sup> Because of the ability for proliferation, and if cortical dementias have an astrocytic cause, astrogenesis could lead to an understanding of prevention through regeneration, or a disruption of astrogenesis could be involved in disease etiology. Here we review human cortical astrocytes and conditions that lead to cortical astrogenesis, with a special focus on neurodegenerative disease.



## Types of Human Cortical Astrocytes

**Protoplasmic astrocytes.** Human cortical astrocytes are pleomorphic, and the morphology and density of astrocytes differ between and within brain regions.<sup>32,33</sup> Human protoplasmic astrocytes are the most abundant astrocyte in the human cortex and reside in layers II–VI.<sup>34</sup> Astrocytes in the human cortex increase their cell body size and projections in response to perturbations in the extracellular and neuronal environment, and the result is the upregulation of intermediate filament glial fibrillary acidic protein (GFAP), which is a distinguishing factor of astrocyte classification.<sup>35</sup> The hypertrophic morphology is termed reactive astrogliosis and results in local proliferation of a subset of protoplasmic astrocytes.<sup>36</sup>

Contrary to the appearance associated with a GFAP-immunostained astrocyte, or those drawn based on classic silver stain, dye injection studies reveal their shape and processes to have a “bushy” appearance.<sup>30</sup> These bushy-like endfeet and extensive projections allow protoplasmic astrocytes to make connections with 270,000–2 million synapses in the human brain compared to 20,000–120,000 synapses in the rodent models.<sup>37</sup> Human cortical protoplasmic astrocytes form independent signaling domains with nonoverlapping territories, are the most complex compared to other primates and rodents, have 2.55 times larger diameter than the rodent, equating to 16.5 times great volume, and signal via calcium wave over 5 times faster.<sup>37</sup> Protoplasmic astrocytes also make contact via their end feet with perivascular processes.<sup>38,39</sup>

Human cortical protoplasmic astrocytes communicate via calcium signaling through end feet gap junctions to other astrocytes adjacent to their domains.<sup>37</sup> The communication results in the release of transmitter to the extracellular space and can happen in response to extracellular transmitter communication from neurons and other astrocytes.<sup>40,41</sup> It has been demonstrated that the transmitters ATP and glutamate stimulate calcium signaling in human cortical astrocytes.<sup>37</sup>

**Interlaminar astrocytes.** In primates, a distinct astrocyte cell type with a stellate morphology has been described in layer I—the molecular layer of the cortex—that does not appear in other mammals.<sup>42–45</sup> In the prosimian *Microcebus murinus*, its length is shorter and it is found in fewer numbers, suggesting the increasing importance of the interlaminar astrocyte in primate species with a more recent common ancestor with humans.<sup>46</sup> However, in other new- and old-world monkeys, including humans, their numbers are similar. Interlaminar astrocytes send a process that extends into and ends in layers III and IV from the molecular layer, making contact with other cells and blood vessels, which does not respect specific domains as is seen with protoplasmic astrocytes.<sup>37,46</sup> Calcium wave signaling was shown to transmit down this projecting process for long distance communication.<sup>37</sup>

**Varicose projection astrocytes.** The least studied and most recently discovered type of astrocyte in the human cortex resides in layers V–VI and extends long processes up to

layers III, IV, and V, making numerous cellular contacts and blood vessel projections.<sup>37</sup> Varicose projection astrocytes are completely unique to the hominid and have not been seen in other primates or in rodents.<sup>32,37</sup> The function of these cells, much similar to that of interlaminar astrocytes, is uncertain at the moment. However, when human glial progenitor cells were grafted into the forebrains of mice, they differentiated into interlaminar and varicose projection astrocytes and had increased learning and memory capabilities compared to murine astrocytes. This was evidenced behaviorally, as well as on the cellular level, through neuronal long-term potentiation studies.<sup>47</sup> Although the tauopathies, synucleinopathies, and other cortical dementias appear to be more prevalent in humans than other mammals,<sup>48</sup> interlaminar and varicose projection astrocytes have not been researched in degenerative disease and are interesting avenues for future studies. Because of the unique primate nature of interlaminar and varicose projection astrocytes, experimental studies in rodent models described here specifically consider cortical protoplasmic astrocytes, which are the only subtype of astrocyte currently described in the rodent cortical gray matter. In human and primate experiments, protoplasmic astrocytes are also the predominant cell discussed, unless otherwise indicated.

## Cortical Reactive Astrogliosis and Astrogenesis

**Protoplasmic astrocytes.** Reactive astrogliosis is typically defined as the change in morphology of astrocytes and subsequent upregulation of proteins involved in neuroprotection near injury, ischemia, or neurodegeneration.<sup>49</sup> However, due to the nature of the cortex, astrocytes are consistently responding to complex changes and subjected to stimuli that can produce astrogliosis, so even subtle perturbations in the nervous system are likely to produce a local reaction.<sup>50</sup> This can happen along a graded continuum of a series of responses, where it is unknown what series of signals produce local astrogliosis.<sup>51</sup> Reactive astrogliosis has most been studied after injury and lesion, and during these insults, astrocyte proliferation is observed, a process traditionally termed astrogliosis.<sup>2</sup>

Reactive astrogliosis in injury and disease states was originally considered detrimental because in severe cases a GFAP+ glial scar forms and sends signals that inhibit neurite outgrowth;<sup>52</sup> however, it is now established to play a neuroprotective role.<sup>6</sup> Proliferating astrocytes appear to be crucial to the recovery after injury, as transgenic experiments that eliminated astrocyte proliferation demonstrated a larger lesion-reduced scar formation and persistent blood-brain barrier dysfunction.<sup>53</sup> In vitro, reactive astrocytes formed neurospheres and have shown stem cell potential.<sup>1,54</sup> In vivo, the cortex appears to foster a gliogenic environment, as ectopic grafting of cells with immature neuronal precursor markers into the cortex reverted back to oligodendrocyte or astrocyte phenotypes.<sup>55</sup>

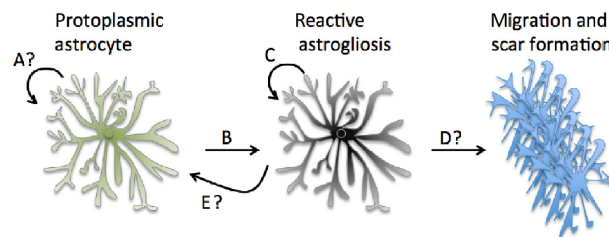
An increased expression of GFAP is typically used as a marker of cortical reactive astrogliosis, but this is not indicative

of proliferation, but rather an upregulation of the protein in response to insult.<sup>49</sup> In milder insults and distal to the injury site, astrocytes can exhibit reactivity, which has been termed “isomorphic,” and are predominantly devoted to promoting neurite growth, synaptogenesis, and neuronal modeling.<sup>49</sup> “Anisomorphic” astrocyte morphology with overlapping domains and proliferation appears to occur in more severe injury,<sup>49</sup> although proliferation occurs along a continuum, with graded increases from mild to severe, and the exact molecular stimulus is currently unknown.<sup>50</sup>

Genetic lineage tracing demonstrated that reactive protoplasmic astrocytes in the cortex began to proliferate within 3–5 days of injury, with half of them reentering the cell cycle up to a week after injury. Known progenitor cell markers, such as nestin, DSD1 proteoglycan, and CD15, are upregulated, and protoplasmic astrocytes isolated from injured tissue also produce neurospheres in vitro.<sup>1,56,57</sup> After controlled cortical impact, it was shown that astrocytes proliferate at all three stages after injury, with 70% of proliferating cells staining for GFAP.<sup>58</sup> In hypoxic-ischemic stroke model of the cortex, GFAP colocalized with bromodeoxyuridine (BrdU), demonstrating astrocyte proliferation in this experimental model as well.<sup>59</sup> Astrocytes have been shown to increase Notch-1 expression to induce proliferation, which reduced proliferation when blocked.<sup>60</sup> GFAP-CreER-Notch-1-cKO mice also exhibited a defect in astrocyte proliferation in adults after ischemic injury.<sup>61</sup>

**Proliferation vs. astrogenesis.** Although certain cortical reactive astrocytes can proliferate, it remains to be seen whether the progeny can differentiate into a nonreactive physiological state. Recently, it was shown through genetic fate-mapping studies and live imaging that 45% of proliferating protoplasmic astrocytes after stab wound in the mouse cortex had soma in direct contact with blood vessels. With unique juxtaposition on blood vessels, the cells proliferate and maintain the bushy morphology of functional astrocytes in response to injury.<sup>2</sup> The cells divided only once, creating two daughter cells after injury, and can function for many weeks after division, indicating astrogenesis after proliferation, even while retaining the bushy reactive morphology. Interestingly, these cells were typically further from the injury site. Cells adjacent to the stab lesion did not appear to proliferate. Instead, they became reactive and exhibited a polar morphology, by extending long processes to the injury site.<sup>2</sup> Proliferating astrocytes also did not seem to contribute to the glial scar, indicating in this study that GFAP+ cells in the scar must be derived from another source (Fig. 1).<sup>2</sup>

**NG2 cells (syntactocytes).** In vitro studies demonstrate that two other cortical cell types, namely neural/glial antigen 2 (NG2) proteoglycan-expressing cells and blood vessel lining pericytes, can differentiate into astrocytes.<sup>62,63</sup> In vivo studies on the ability for pericytes to differentiate into astrocytes, however, have not been studied in the cortex. Additionally, NG2 cells in the cortex were long considered to be a subset



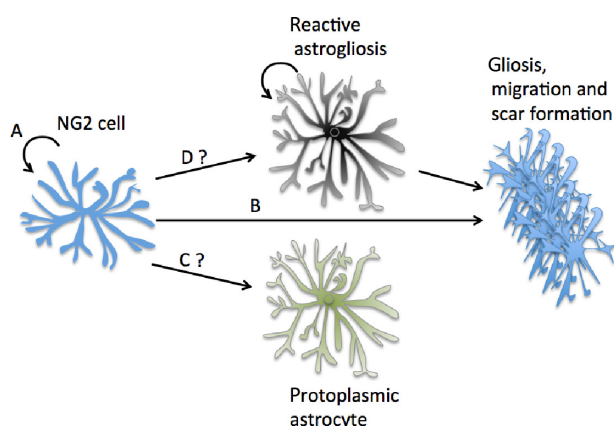
**Figure 1.** Local astrogenesis from protoplasmic astrocytes. (A) It is unclear to what extent local cortical astrogenesis occurs in the healthy brain. (B) Reactive astrogliosis occurs as an astrocytic response to changes in the extracellular environment—in some cases this can lead to proliferation in C. (D) It is generally believed that protoplasmic astrocytes contribute to the glial scar when reactive in injury, although recent evidence indicates that the GFAP+ cells in the scar may derive from a different source.<sup>2</sup> (E) Recent evidence indicates that reactive astrocytes can retain their physiological function after proliferation, although the molecular process and time course are still unclear.<sup>2</sup>

of astrocytes because of their morphology and functionality.<sup>64</sup> However, it is now known they are not a subset of astrocytes, as the mature NG2 cell is functionally different and does not express many typical astrocyte markers.<sup>65</sup> NG2 cells are also the main oligodendrocyte precursor in the cortex, which is distinct from mature cortical astrocyte stem cell function in vivo.<sup>65</sup> They have a long cell cycle of one month in the adult human cortex and consistently regenerate to contribute to the oligodendroglial pool.<sup>66,67</sup>

NG2 cells also proliferate after injury and have been shown in severe cases to differentiate into astrocytes in the cortex and contribute to the glial scar and neuroprotection.<sup>68–71</sup> NG2 cells are the second type of cell to proliferate after injury to the brain,<sup>66</sup> after microglia, which are recruited to the injury site by astrocytes.<sup>72</sup> After recent studies on astrocyte proliferation demonstrated they did not incorporate into the scar in stab wound studies, it is possible NG2 progenitors differentiate into scar-forming GFAP+ cells and contribute more than thought to the scar. Early genetic fate-mapping studies also confirmed that NG2 cells differentiate into astrocytes in the cortex after injury.<sup>73</sup> Immunohistochemical studies showed that 5–8 days after injury, 20% of GFAP+ cells colocalize with NG2, indicating that NG2 progenitors differentiate into reactive astrocytes under certain conditions.<sup>69</sup> Another study showed that in the cortex, within a week after injury, a small population of NG2 cells will express vimentin and nestin, two immature astrocyte markers.<sup>71</sup>

However, there is some conflicting evidence for their astrocyte lineage, as other studies demonstrated that NG2 cells do not appear to be proliferating into astrocytes.<sup>74</sup> In the spinal cord of amyotrophic lateral sclerosis (ALS) mice, it was determined through fate-mapping studies that NG2+ cells were committed to an oligodendrocyte fate postnatally.<sup>75</sup> However, another study showed that genetic fate-mapping studies indicated that NG2 cells could become astrocytes in postnatal development.<sup>76</sup> Further studies demonstrated that a subset of





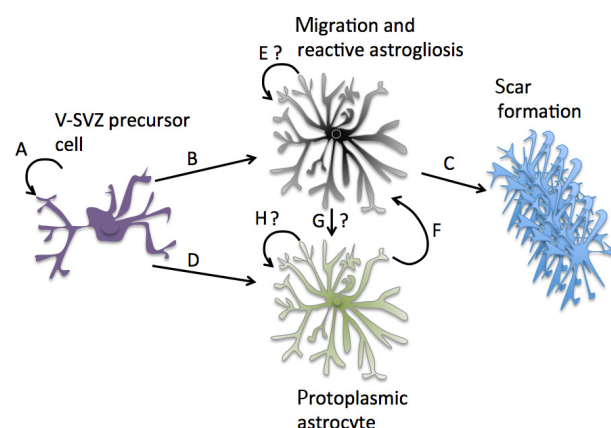
**Figure 2.** NG2 cells and cortical astrogenesis. NG2 cells divide monthly as indicated in **A** and are known to become reactive and contribute to the glial scar in **B**. **(C)** Many early lines of evidence on NG2 cells pointed to them as an astrocyte precursor cell in the adult cortex. Recent fate-mapping studies have shown that may not be the case.<sup>74</sup> **(D)** It is also unsure whether they can become reactive astrocytes under severe conditions as was previously believed and then proliferated.<sup>74</sup>

NG2 cells can indeed differentiate into protoplasmic astrocytes postnatally; however, by postnatal day 60, no new astrocytes were born from NG2 cells.<sup>77</sup> Although most evidence points to astrocyte and NG2 cell proliferation in injury conditions,<sup>78</sup> there is currently no indication that NG2 cells can become mature functional protoplasmic astrocytes (Fig. 2).

**Adult stem cells in germinal layers.** While experimental evidence indicates that mature cortical protoplasmic astrocytes can proliferate in certain conditions, one question is whether it is possible that cells can migrate from germinal niches, such as the ventricular–subventricular zone (V-SVZ)<sup>79,80</sup> or the subgranular zone (SGZ) in the hippocampus to contribute to the cortical astrocyte population. GFAP+ adult neural stem cells with astrocyte properties in the subventricular zone of mammals have been shown to differentiate into neuronal and astrocytic precursors and migrate to other areas of the brain, most notably the olfactory bulb.<sup>81–85</sup> However, focal ischemia of the striatum adjacent to the V-SVZ produced mainly glial lineages, with 60% astrocytes from neural stem cells.<sup>86</sup> Similarly, in rodents, GFAP+ astrocytes in the SGZ of the hippocampus can give rise to new functioning neurons and mature astrocytes.<sup>87</sup>

After birth, in the human brain prior to 18 months, proliferating cells in the V-SVZ migrate to the prefrontal cortex with an astrocytic fate instead of a neuronal one, before subsiding, with astrocytes proliferating in the cortex locally afterward.<sup>88</sup> In piglets by postnatal day 7, it was seen that few, if any, proliferating V-SVZ cells colocalized with immature neurons.<sup>89</sup>

After cortical injury, nestin+ cells that did not express GFAP were shown to migrate and become new ipsilateral astrocytes to the injury site.<sup>90</sup> Also, in normal conditions, V-SVZ fate-mapped nestin+ cells become astrocytes in the



**Figure 3.** V-SVZ adult stem cells and cortical astrogenesis. V-SVZ adult stem cells are regenerating cells **(A)** that have recently shown to proliferate into protective reactive astrocytes after injury **(B)** and contribute to the glial scar in the cortex **(C)**.<sup>92</sup> **(D)** It is also known that they can contribute to adult astrogenesis. However, it is unclear whether the reactive astrocytes they produce can proliferate locally **(E)**, although this proliferation likely occurs if derived locally from a mature astrocyte **(F)**. It is also uncertain whether reactive astrocytes produced by the V-SVZ can become mature protoplasmic astrocytes **(G)**, which may also be able to proliferate **(H)**.

corpus callosum but did not appear to contribute to astrogenesis in the cortex.<sup>91</sup>

Interestingly, it was recently shown with tamoxifen-induced nestin-Cre<sup>tm</sup> 4 lineage tracing that the majority of cells that contribute to a cortical injury site are produced through astrogenesis, with cells deriving from a V-SVZ lineage.<sup>92</sup> Some cells from the V-SVZ contributed to the glial scar and were also high thrombospondin-producing cells, a protein released by astrocytes known to induce synaptogenesis (Fig. 3).<sup>14</sup> KO mice for thrombospondin 4 caused alterations in the glial scar and increased microvascular hemorrhage.<sup>92</sup>

In the hippocampus, it is known that neural precursor cells in the SGZ contribute to the mature astrocyte population in CA1 under normal physiological conditions and that this process is disrupted in injury and disease.<sup>93</sup> However, although of interest because of known hippocampal degeneration in AD, the evidence is scant for astrocyte proliferation from the SGZ to the cortex.<sup>94</sup> In aging mice, division happened less frequently, and GFAP-expressing cells began to exhibit characteristics of reactive astrogliosis.<sup>95</sup> Immediately after injury to the cortex, astrocyte proliferation occurred locally in the hippocampus without migration to the cortex.<sup>96</sup>

### Cortical Astrogenesis in Other Conditions

**Aged and normal healthy cortex.** It appears that the astrocyte capability of reactivity and proliferation is inherent to astrocytic cell function. During development, astrocytes derive from radial glial cells, but postnatally, in the mouse cortex, they proliferate locally and incorporate into functional units with defined astrocyte regions.<sup>97</sup> Because of the

astrocyte ability to respond to even slight perturbations in the parenchyma,<sup>50</sup> studies on cortical astrogenesis in “normal” healthy adult brains or aged cortex might reveal the mechanisms of astrocytic proliferation.

Early studies of aged human brains are somewhat conflicting because gray matter human astrocytes were designated as “fibrous” astrocytes in many cases. Fibrous astrocytes currently refer to astrocytes residing in white matter tracts, but before this designation, protoplasmic or interlaminar astrocytes were determined to be “fibrous” in some studies if they were labeled immunohistochemically with GFAP.<sup>98</sup> It is believed that astrocytes undergo a change in morphology in aging, and originally, it was thought that astrocyte reactivity was increased in aging, as well as consequential astrogenesis, whereby an increase was seen on an average of >20% of astrocytes in the cortex of aged brains.<sup>99,100</sup> Furthermore, in the aged rat cortex, a 20%–22% increase in astrocytes and pericytes was shown.<sup>101</sup>

However, it must also be remembered that an increase in GFAP expression of cortical protoplasmic astrocytes is not indicative of proliferation<sup>102</sup> but traditionally a marker of reactive astrogliosis.<sup>6</sup> One study analyzed the brains of several aged controls with no neurodegenerative disease diagnosis and noticed an increase in the GFAP expression of the cortex, in both the molecular layer and cellular layer.<sup>103</sup> As the interlaminar astrocyte is unique to primates in the molecular layer, it is likely that interlaminar astrocytes undergo reactive astrogliosis in response to aging. Additionally, in cellular layers II–VI, increased GFAP expression was associated with perivascular location and typically, in duplicate, indicating possible proliferation in aging.<sup>103</sup> In rats, increased GFAP expression was also noticed in the aging cortex.<sup>104</sup>

In the entorhinal cortex of aged mice, an area that degenerates early in AD a decrease in GFAP expression and astrocyte atrophy was observed.<sup>105</sup> Also, in another human study of female brains, aged 65–75, 76–85, and 94–105 years of age, it was observed that there was no change in neuron or astrocyte numbers.<sup>106</sup> Additionally, other sources indicate no increase in the amount of astrocytes in the cortex.<sup>107</sup> In rats, an electron microscopic study concluded that there was an increase in the number of astrocytes in aging animals compared to controls.<sup>108</sup> Nonneuronal cells stained with cresyl violet were also increased in the parietal cortex of aged rats compared to controls.<sup>109</sup>

Studies of nonnervous system origin cancer patients injected with BrdU demonstrated that new cells formed in the cortex were nonneuronal with a small subset colocalizing with GFAP <0.5 cell/mm<sup>3</sup>, indicating the prevalence of astrogenesis in the noninjured nondegenerating adult cortex.<sup>110</sup> Neurons, however, did not colocalize with BrdU, indicating that new neurons were not formed in the cortex in the lifespan of the organism and that proliferation was strictly glial.<sup>110</sup>

In rhesus monkeys, neuronal cell loss was not observed in the cortex of aged monkeys, and astrocytes had exhibited an

increase in cellular inclusions. The older monkeys had significant memory impairment compared to the younger monkeys.<sup>111</sup> However, in subsequent studies, they noticed that only microglia increased in numbers with aging.<sup>112</sup> Although many studies have indicated that increased GFAP staining correlates with age, indicating an increase in astrocyte reactivity,<sup>113</sup> studies on astrogenesis in healthy human cortex are few.

#### **Learning, exercise, and environmental enrichment.**

Environmental enrichment has been known to increase cell division in the adult brain because a study by Altman and Das in the 1960s demonstrated a significant increase in gliogenesis in the brains of rats.<sup>114</sup> The cell type was not determined, and they noticed increased cell division in all the areas of the white matter of the coronal radiations. Cell division occurred in the gray matter as well, but this was not statistically studied.<sup>114</sup>

The hippocampus is currently the region of the brain with the most evidence for increased astrogenesis in environmental enrichment conditions, where astrocytes from the SGZ proliferated into mature astrocytes in the CA1 region.<sup>93</sup> Cells were shown to be mature and distinct from the GFAP+ progenitor cells where they arose in the SGZ.<sup>115</sup> Cells in the SGZ that are GFAP+ can also differentiate into neurons,<sup>116</sup> and this is increased in environmental enrichment and learning conditions.<sup>117</sup> GFAP expression and increased size and complexity of astrocytes were seen in the dentate gyrus after physical activity and environmental enrichment.<sup>118</sup>

There is also evidence for cortical astrogenesis in environmental enrichment, as in the motor cortex of mice, a noticeable increase in astrogenesis was observed, without an increase in oligodendrocytes and with no new neurons formed.<sup>119</sup> Additionally, operant conditioning tasks showed that astrogenesis occurred in the prefrontal cortex and that learning maintained cell survival, whereas if learning did not occur, new cells were not maintained.<sup>120</sup> Voluntary exercise also resulted in a 3× increase in astrogenesis compared to normal controls in the medial prefrontal cortex of mouse brains.<sup>121</sup> Although the lineage of the proliferating astrocytes in the prefrontal cortex of the mouse brain has not yet been studied, they appear to be from local progenitors or originating from cells in the V-SVZ.

**Cortical spreading depression.** Studies have shown that cortical spreading depression (CSD) can cause the proliferation of astrocytes in cortical regions. This is preceded by cortical spreading depolarization, which is associated with migraine, stroke, and epilepsy and results in the excitation spread of coordinated neuronal firing.<sup>122</sup> CSD results in an increase in a number of dividing cells that coexpress GFAP.<sup>123–126</sup> Cortical brain slice preparation demonstrated the origin of the cells were NG2 cells differentiating into astrocytes.<sup>127</sup> In the entorhinal cortex, a robust increase in cell proliferation to CSD remained astrogenic, and no subsequent cortical neurogenesis was observed.<sup>128</sup> CSD shifted the relative frequencies of glial cells from NG2 cells to astrocytes and microglia.<sup>128</sup> Nestin+ astrocytes were also increased after CSD in the cortex.<sup>97</sup>



## Cortical Astrocytes and Neurodegeneration

**Tauopathies and amyloid- $\beta$ .** Reactive astrogliosis as described by hypertrophy and subsequent proliferation is found in chronic neurodegenerative lesions.<sup>6,49,50</sup> In human, similar to what was studied in aging brains, where an increase in GFAP+ expression of protoplasmic astrocytes was noticed in cortical layers II–VI after age 70, a much larger increase in GFAP+ cells was seen, which was greater than four times in the cellular layer of patients diagnosed with AD compared to age-matched controls.<sup>98</sup>

Amyloid precursor protein (APP) when cleaved by beta-secretase 1 (BACE-1) and gamma secretase produces amyloid- $\beta_{1-40}$  and amyloid- $\beta_{1-42}$ , peptides that accumulate in amyloid plaques in neurodegenerative disease.<sup>129</sup> Additionally, amyloid- $\beta_{1-42}$  is closely associated with disease<sup>130</sup> and has been shown to preferentially stimulate astrogenesis from human embryonic neural stem cells in vitro,<sup>131</sup> while BACE-1 null mice show diminished astrogenesis in the hippocampus.<sup>132</sup> In vitro, the amyloid- $\beta_{1-42}$  peptide treatment of postnatal primary mouse astrocytes increased proliferation<sup>133</sup> and was shown to disrupt calcium signaling between astrocytes, which is also diminished in disease progression.<sup>134,135</sup> APP has been shown to stimulate astrogenesis in development as well.<sup>136</sup>

Amyloid- $\beta_{1-40}$  and amyloid- $\beta_{1-42}$  are cleared from the extracellular space by astrocytes through the glymphatic system.<sup>17</sup> It was observed that astrocytes surrounding plaques increase the expression of GFAP and vimentin; however, in GFAP and vimentin KO mice, the plaque load was not diminished, but lysosomal and inflammation genes increased expression.<sup>137</sup>

Importantly, researchers observed that proliferative circumferential reactive astrogliosis around amyloid- $\beta$  plaques correlated with cognitive scores in disease.<sup>138</sup> Synapse loss in the cortex also occurs early on in disease and correlates with cognitive decline,<sup>7-9</sup> and astrocytes contribute to the regulation of synaptogenesis.<sup>15</sup> This lack of circumferential astrogliosis also correlated with apolipoprotein E  $\epsilon 4$  genotype, a known genetic precursor for late-onset AD,<sup>138</sup> which is a risk factor after early life incidence of head injury, another known stimulator of astrogenesis.<sup>139</sup> Astrocytes are the predominant apolipoprotein E-producing cell in the cortex,<sup>140</sup> and the protein appears to be involved in cholesterol transport via lipid rafts, a contributor to synaptogenesis.<sup>141</sup>

In the TgCRND8 mouse AD model, it was observed that GFAP+ cells colabeled with BrdU in aged mice, indicating a proliferative response.<sup>142</sup> However, when proliferating cells in another mouse model of AD, the APPswe/PS1dE9 (APPPS1) transgenic mice were studied; microglia were the main proliferating cell type. GFAP+ reactive astrocytes were not proliferative around the plaque, compared to those in injury, where reactive astrocytes are prevalent and begin to proliferate, as the severity of the injury increases.<sup>143</sup>

In vitro, neurospheres indicative of stem cell properties have been produced from astrocytes after injury in APPPS1

mice. Also, it has been shown that 2.7% of cortical proliferating cells were astrocytes, which account for only about 1.1% of the astrocytes in the cortex.<sup>144</sup> Many of the cells were microglia and NG2 cells. Many astrocytes not proliferating also produce immature cell markers, such as nestin, DSD1, and tenascin-C, which are upregulated in reactive astrocytes.<sup>144</sup> However, it appears that sonic hedgehog signaling is responsible for astrocyte proliferation from reactive astrocytes.<sup>144</sup>

Glial atrophy has been shown in the cortex of the APPPS1 AD mouse.<sup>145</sup> In AD transgenic mice, the hippocampus exhibited extensive reactive astrogliosis, but not the entorhinal cortex where astrocyte atrophy was observed, which is one of the areas of the brain to exhibit selective early vulnerability in AD.<sup>146</sup> Additionally, astrocyte atrophy was observed in medial prefrontal cortex in AD transgenics.<sup>147</sup>

Additionally, neurofibrillary tangles formed as a result of hyperphosphorylated tau protein aggregation are observed in AD neurodegeneration and can occur in cortical tauopathies independent of plaque formation.<sup>148</sup> In other tauopathies, such as frontotemporal dementia, astrocyte apoptosis preceded neuronal apoptosis in the disease progression.<sup>149</sup> In a transgenic model of tauopathy, there was an age-related increase in tau accumulation in astrocytes, which is similar to what is seen in neurons in disease.<sup>150</sup> A reduction of astrocyte glutamate transporter 1 was observed in corticobasal degeneration, and a tau mouse model from the GFAP promoter demonstrated similar vascular defects and neurofibrillary tangle formation in disease states.<sup>151</sup> The hyperphosphorylation of tau, and accumulation within astrocyte end feet processes was also observed to contribute to vascular defects in corticobasal degeneration and progressive supranuclear palsy.<sup>152</sup> In many cases, AD and other tauopathies can be thought of as a cerebrovascular disease and have been considered as such, where it has been estimated that as many as 84% of cases show both morphologies.<sup>153</sup>

Finally, early on in AD, it is noticed that many genes and proteins involved in cell cycle stimulation are upregulated.<sup>154</sup> This has been considered from a neuronal perspective, with a hypothesis that cell cycle reentry and dysfunction in neurons lead to degeneration.<sup>155,156</sup> However, because of the known proliferative nature of astrocytes, cell cycle biomarkers in astrocytes in disease states provide a future avenue for study.

**Synucleinopathies.** Synucleinopathies are characterized by the accumulation of protein  $\alpha$ -synuclein ( $\alpha$ -syn) in Lewy Bodies inclusions.<sup>157-159</sup>  $\alpha$ -Syn is abundantly expressed at neuronal synapses<sup>160-162</sup> and can be released extracellularly as a possible signaling protein as evidenced by its binding to postsynaptic protein and ability to be transferred to neighboring neurons to form Lewy body inclusions.<sup>163-165</sup> Extracellular  $\alpha$ -syn has also been shown to assimilate in human cortical astrocytes in vivo and in vitro.<sup>166-168</sup> Common synucleinopathies affecting the cortex are multiple system atrophy (MSA), Parkinson's disease dementia, and DLBs.<sup>169</sup>

Genes involved in familial Parkinson's disease, such as Pink1, Parkin, DJ-1, and LRRK2, are specifically expressed



by astrocytes and shown to produce proteins associated with lipid rafts.<sup>170,171</sup> Pink1, Parkin, DJ-1, and LRRK2 were also shown to be involved in cell cycle regulation.<sup>172</sup> Additionally, human cortical astrocytes in culture treated with  $\alpha$ -syn revealed apolipoprotein E redistribution to the cytoplasm and an increase in GFAP+ astrocytes.<sup>167</sup>  $\alpha$ -Syn signaling to astrocytes at the synapse was also shown to be increased in the songbirds developing song control system and demonstrates a possible involvement in neuroplasticity.<sup>160</sup>

In midbrain regions affected early in Parkinson's disease, reactive astrogliosis in the substantia nigra was similar to normal control tissue, whereas in another synucleinopathy, multiple system atrophy, reactive astrogliosis was increased in the substantia nigra.<sup>173</sup> However, in the frontal cortex in both Parkinson's disease and multiple system atrophy, an increase in reactive astrogliosis as marked by GFAP, vimentin, and heat shock protein-27 immunoreactivity was observed.<sup>173</sup> Additionally, astrocyte and microglia marker YKL-40 were significantly reduced in the cerebral spinal fluid of patients with synucleinopathies (PD, MSA, and DLB) compared to tauopathies, where the reduction was significant compared to controls but higher than the synucleinopathies.<sup>174</sup>

A model of MSA demonstrated that  $\alpha$ -syn can induce reactive astrogliosis in the frontal and visual cortex of human brain via astrocyte proximity to accumulated  $\alpha$ -syn inclusions.<sup>175</sup> In other GFAP and vimentin expression studies, it was observed that, unlike that in AD, cortical reactive astrogliosis does not correlate with cognitive decline in Parkinson's disease dementia compared to normal controls.<sup>176,177</sup> In vivo, it appears that there is early dysfunction in astrocytes in disease progression, as there is an indication that cortical protoplasmic astrocytes become non-reactive and susceptible to  $\alpha$ -syn accumulation while recruiting microglia to attack the affected neurons.<sup>178</sup> Selective expression of A53T mutant  $\alpha$ -syn in astrocytes also resulted in aggressive disease progression in mice.<sup>179</sup>

Recently, in human neuropathological studies  $\gamma$ -synuclein ( $\gamma$ -syn), another member of the synuclein family, was shown to be expressed in cellular inclusions along with  $\alpha$ -syn.<sup>180</sup>  $\gamma$ -Syn is upregulated in glioblastomas<sup>181</sup> and is known to be involved in cell cycle regulation.<sup>182</sup> An increase in the expression of  $\gamma$ -syn was also seen along with  $\alpha$ -syn in the cerebral spinal fluid of patients diagnosed with synucleinopathy and vascular disease,<sup>183</sup> and a mouse model overexpressing  $\gamma$ -syn demonstrated widespread neuropathy.<sup>184</sup>

## Conclusion

Although the general notion is that astrocytes achieve a quiescent mature cell fate in adulthood, the physiology of astrocytes in the brain during neuronal communication and neuronal dysfunction allows for them to be dynamic in their response, whereby they undergo reactive astrogliosis and proliferation to protect the neuronal environment. The exact type of perturbations on the molecular level that stimulate proliferation is currently unknown. It is also unknown to what extent over

time reactive astrocytes can then resume normal function after proliferation.

Additionally, fate-mapping studies have provided clearer observations on the lineage of cells proliferating in the cortex in disease and injury states, but the evidence is still murky. The contribution and function of cells arising from local proliferation is yet to be determined. Also, the function in early disease states of reactive astrocytes, in addition to biomarkers for the manipulation of astrocytic mechanisms in disease, will be useful. In particular, due to upregulated cell cycle markers and for cell replacement, neurogenesis has been studied in injury and disease cause and prevention; however, because of the inherent proliferative abilities of adult astrocytes compared to neurons, as well as neuroprotective functions, studies on astrogenesis could provide insights into disease onset.

Because much of the early research in neurodegenerative disease has focused on the response of astrocytes to neuronal degeneration, there are many avenues to consider for the study of astrocyte involvement in the cause of degenerative disease. For instance, it is now becoming clear that astrocyte dysfunction can lead to synaptic loss, neurodegeneration, and protein accumulation in the form of Lewy bodies, neurofibrillary tangles, and amyloid plaques. Therefore, an exploration of astrogenesis, in the normal aging brain and neurodegenerative disease, could provide fruitful studies on the cause and prevention of degenerative diseases of the brain.

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## Author Contributions

Wrote the first draft of the manuscript: TCM. Contributed to the writing of the manuscript: TCM and AOK. Agree with manuscript results and conclusions: TCM and AOK. Jointly developed the structure and arguments for the paper: TCM and AOK. Made critical revisions and approved final version: AOK. All authors reviewed and approved the final manuscript.

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