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REVIEW ARTICLE

Regionally encoded functional heterogeneity of astrocytes in health and disease: A perspective

Benjamin E. Clarke[1,2] | Doaa M. Taha[1,2] | Giulia E. Tyzack[1,2] | Rickie Patani[1,2]

[1]Department of Neuromuscular Disease, UCL Queen Square Institute of Neurology, Queen Square, London, UK

[2]The Francis Crick Institute, London, UK

Correspondence
Rickie Patani, Department of Neuromuscular Disease, Institute of Neurology, University College London, London WC1N 3BG, UK.
Email: rickie.patani@ucl.ac.uk

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Abstract

Increasing evidence has suggested that astrocytes demonstrate striking regionally allocated functional heterogeneity. Here, we discuss how this spatiotemporally encoded diversity determines the astrocytic phenotype along a finely grained spectrum from neuroprotective to deleterious states. With increasing recognition of their diverse and evolving roles in the central neuraxis, astrocytes now represent a tractable cellular target for therapies aiming to restore neural circuit integrity in a broad range of neurodegenerative disorders. Understanding the determinants of astrocyte physiology along with the true extent of heterogeneity in their regional and subregional functions will ultimately inform therapeutic strategy in neurodegenerative diseases.

KEYWORDS

aging, astrocytes, functional heterogeneity, neurodegeneration, regional identity

1 | INTRODUCTION

Astrocytes have a myriad of well-documented roles in neuronal homeostasis including metabolic support and synapse regulation (Vasile, Dossi, & Rouach, 2017). In response to stimuli, astrocytes can undergo context-dependent changes in their gene expression in a process termed reactivity, which can have protective or detrimental effects on surrounding neurons (Anderson et al., 2016; Liddelow et al., 2017). In several neurodegenerative diseases, both the loss of astrocytic support to neurons and the transformation of astrocytes to a toxic reactive state have been implicated in contributing to pathological mechanisms (Liddelow & Barres, 2017; Phatnani & Maniatis, 2015). The taxonomy of neurological disorders is heavily biased toward the loss of regionally defined and subtype-specific neurons. Yet the role of juxtaposed astrocytes in this process, which form neuroglial units, is often overlooked.

Astrocyte heterogeneity can be considered from different perspectives including (a) reactive state, (b) regional identity, and (c) age-related changes. Astrocyte reactive state has been comprehensively reviewed elsewhere (Ben Haim, Carrillo-de Sauvage, Ceyzériat, & Escartin, 2015; Escartin, Guillemaud, & Carrillo-de Sauvage, 2019; Liddelow & Barres, 2017). Accumulating evidence suggests that astrocytes possess a unique regional identity, although the granularity of this positional specification is not yet fully resolved. Given the vast and well-established nature of neuronal subtype diversity, astrocyte specialization may mirror this to a greater degree than currently suspected. Astrocyte regional heterogeneity may be considered at morphological, molecular, and functional levels, with evidence for both interregional and intraregional differences (Khakh & Deneen, 2019). Here we provide a perspective on how the attribute of regional identity affects astrocyte function and how regional heterogeneity of astrocytes might contribute to age-related vulnerability in a broad range of neurodegenerative disorders.

2 | THE DEVELOPMENTAL "LOGIC" OF REGIONAL IDENTITY IN ASTROCYTES

An important question concerning astrocytic heterogeneity is *when* such nuanced features arise. One scenario is that this is "hard-wired"

Benjamin E. Clarke and Doaa M. Taha contributed equally to this study.

regional encoding during early embryonic stages, a concept that is well established for neurons (Jessell, 2000). Alternatively, the unique specialization of astrocytes may be acquired through signals from the relevant neuronal circuits that they will ultimately subserve. Indeed, both developmental transcriptional programs and later refinement by local environmental cues are likely to each play important roles in the establishment and maintenance of astrocyte functional heterogeneity. The relationship between developmental origin and final positional identity of astrocytes is also noteworthy here as these cells have significant migratory capacity in early development, which is then limited upon aging (Jacobsen & Miller, 2003). This reinforces the likely importance of local cues refining astrocytic regional identity. An example of this is the effect of neuronal Sonic Hedgehog (SHH) signaling, which affects astrocyte protein expression and identity (Farmer et al., 2016; Hill et al., 2019). In both the cortex and hippocampus, SHH signaling affected the expression of astrocytic potassium channel Kir4.1. SHH signaling played an even more pivotal role in the cerebellum, where SHH levels determined the proportion of Bergmann glia and velate astrocytes (Farmer et al., 2016). Developmental transcriptional programs also play a vital role in establishing regional identity of astrocytes - both mouse and human pluripotent stem cells have been directed to regionally identifiable and highly pure astrocyte populations using diffusible morphogens in vitro, at least partly mimicking the in vivo expression profiles of different astrocyte populations (Bradley et al., 2019; Krencik, Weick, Liu, Zhang, & Zhang, 2011). By this method, regionally distinct astrocyte populations have been distinguished both on the dorsoventral and rostrocaudal axes.

The spinal cord is divided into spatially distinct domains, each consisting of a specific neuronal subpopulation. Astrocyte generation within the spinal cord is also temporally regulated, with ventral astrogliogenesis occurring before dorsal (Tien et al., 2012). This arrangement is configured at early stages of development through graded morphogen signaling by the expression of various homeodomain and basic helix–loop–helix (bHLH) transcription factors at the ventricular zone (VZ). In the ventral spinal cord, the expression of Reelin and Slit1 divides white matter astrocytes into three subpopulations, termed ventral astrocyte subtype 1, 2 and 3 (VA1-3) (Hochstim, Deneen, Lukaszewicz, Zhou, & Anderson, 2008) (Figure 1). Through loss and gain of function experiments it was shown that Pax6 promotes the expression of Reelin and suppresses Slit1, thus specifying the VA1 domain (Hochstim et al., 2008). Conversely, Nkx6.1 is expressed in the VA3 domain in the absence of Pax6 and positively regulates Slit1, thus specifying the VA3 domain. In the VA2 domain, both Pax6 and Nkx6.1 are expressed, suggesting that Pax6 repression of Slit1 might be overridden by the presence of Nkx6.1.

Distinct morphological and gene expression profiles have also been found in astrocytes between different cortical layers (Lanjakornsiripan et al., 2018). Fluorescent labeling of the mouse cortex has revealed that astrocyte progenitors colonize the cortex in a

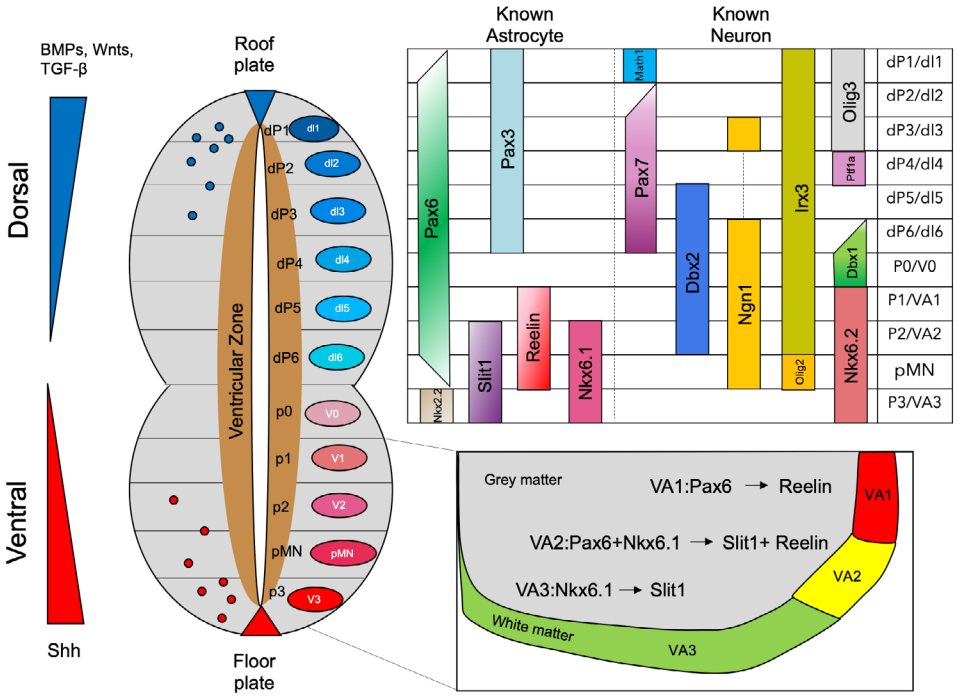

FIGURE 1 Simplified schematic of the developing spinal cord. A gradient of diffusible morphogens from the roof and floor plates determines the expression of certain transcription factors that dictate the positional identity of cells in the spinal cord (Hochstim et al., 2008). BMP, bone morphogenetic protein; Dbx, developing brain homeobox protein; dI, dorsal interneuron; dP, dorsal progenitor; Irx3, Iroquois homeobox protein 3; Math1, mouse atonal homolog 1; MN, motor neuron; Ngn1, neurogenin 1; NKX2.2, NK2 homeobox 2; NKX6, NK6 homeobox; Olig, oligodendrocyte transcription factor; p0, progenitor domain 0; Pax, paired box protein; Ptf1a, pancreas associated transcription factor 1a; Shh, Sonic Hedgehog; Slit1, slit guidance ligand 1; TGF, transforming growth factor; V0, Ventral domain 0; VA, Ventral astrocyte

scattered and non-ordered manner, with extensive intraregional morphological heterogeneity adding further support for the involvement of local environmental factors in specification (Clavreul et al., 2019; García-Marqués & López-Mascaraque, 2013). Interestingly, data from spatial transcriptomics has recently suggested that in the somatosensory cortical grey matter, astrocytes can be approximately divided into three layers across the dorsoventral axis that are distinct from the six neuronal layers (Bayraktar et al., 2020) (Figure 2). Astrocytic regional domains also displayed graded organizational complexity in the cortex through the rostrocaudal axis. Although astrocyte layers did not correspond to neuronal laminae, disruption of neuronal identity by Satb2 knockout also affected astrocytic layering, further suggesting that the establishment of astrocytic regional organization is partly influenced by neuronal signals.

Understanding developmental and temporal mechanisms underlying the ultimate positional specification of astrocytes seems to be a prerequisite to understanding their functional heterogeneity. An important study directly addressed this using the cre-recombinase system driven by the promoters of region-specific transcription factors to trace astrocyte generation along the dorsoventral axis (Tsai et al., 2012). Astrocytes from the p3 progenitor domain, defined by Nkx2.2, remained close to the ventral midline of the spinal cord, while Pax3-expressing astrocytes remained confined to the dorsal spinal cord. The regional identity of astrocytes seems to remain stable once established. Lineage tracing using Ngn3 demonstrated that radial glia generated astrocytes, which remained confined to the intermediate domain 6 months later (Tsai et al., 2012). Interestingly, regional identity remains stable even after mechanical injury or when astrocytes were transplanted ectopically into a different region of the brain of a neonatal mouse (Krencik et al., 2011; Tsai et al., 2012).

3 | REGIONALLY ENCODED FUNCTIONAL HETEROGENEITY IN ASTROCYTES

Over 30 years ago, regionally determined functional heterogeneity of glia was suggested by researchers who studied dopaminergic neurons from the mesencephalon on glial monolayer cultures generated from either the mesencephalon or the striatum. Glial region of origin determined the morphological characteristics of the co-cultured neurons, implicating glia in shaping neuronal architecture (Denis-Donini, Glowinski, & Prochiantz, 1984). The release of diffusible factors was at least partly attributed to the regional heterogeneity of astrocytic support to neurons in experiments where astrocyte conditioned media (ACM) was added to neuronal cultures (Yoshida, Saito, & Katsuki, 1995). Interestingly, ACM from the same region (isotopic cultures) did not always optimally support neuronal survival, as while hippocampal ACM promoted survival of hippocampal neurons more than cortical or hypothalamic ACM, hypothalamic ACM promoted cortical neuron survival more than cortical ACM.

A later seminal study harnessed adult rodent hippocampal stem cells and used their differentiation potential as an assay (Song, Stevens, & Gage, 2002) (Figure 3). In this study, adult stem cells were separately co-cultured with neonatal or adult astrocytes from either the hippocampus or spinal cord. While astrocytes from a non-neurogenic niche—the adult spinal cord—did not support adult hippocampal neurogenesis, neonatal isotopic astrocytes optimally supported neurogenesis from adult hippocampal stem cells compared with adult astrocytes, implicating both spatial and temporal mechanisms in astrocyte mediated neurogenesis.

Regional differences in the morphology of astrocytes have been appreciated for several years, with the identification of astrocyte subtypes in certain regions of the brain such as Bergmann glia in the

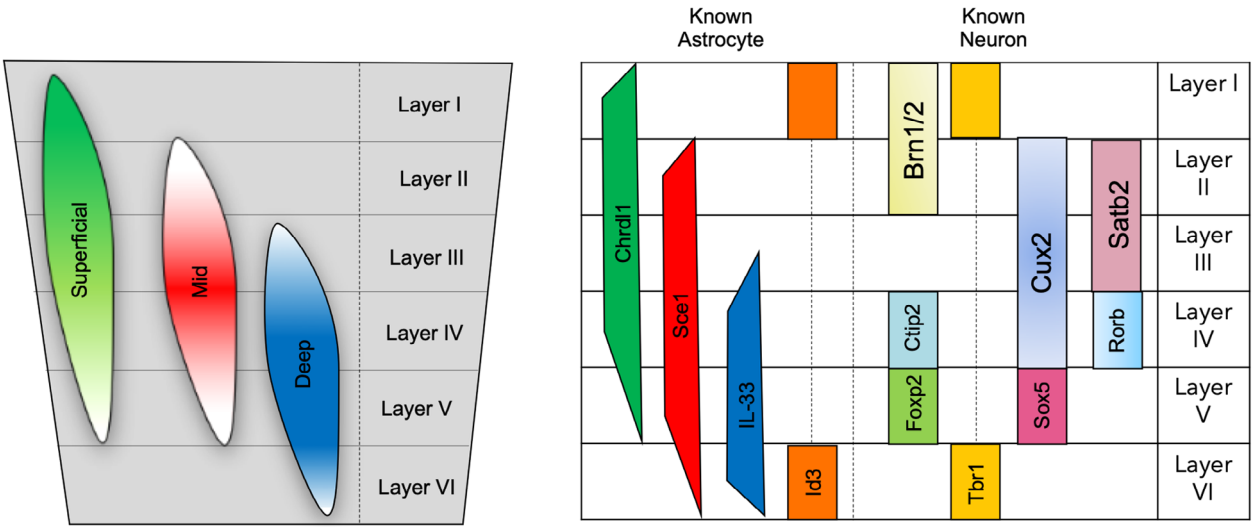

FIGURE 2 Astrocyte positional identity in the somatosensory cortex (Bayraktar et al., 2020). Astrocyte layers in the somatosensory cortex are distinct from neuronal laminae and can be divided into superficial, mid and deep layer astrocytes in the cortical grey matter. Brn1/2, brain-specific homeobox/POU domain protein 1/2; Chrdl1, chordin like 1; Ctip2, COUP-TF-interacting protein 2; Cux2, Cut like homeobox 2; Foxp2, forkhead box protein P2; Id3, inhibitor of differentiation 3; IL-33, interleukin 33; Rorb, RAR-related orphan receptor beta; Satb2, Special AT-rich sequence-binding protein 2; Sce1, SUMO-conjugating enzyme 1; Sox5, SRY-box transcription factor 5; Tbr1, T-box brain transcription factor 1

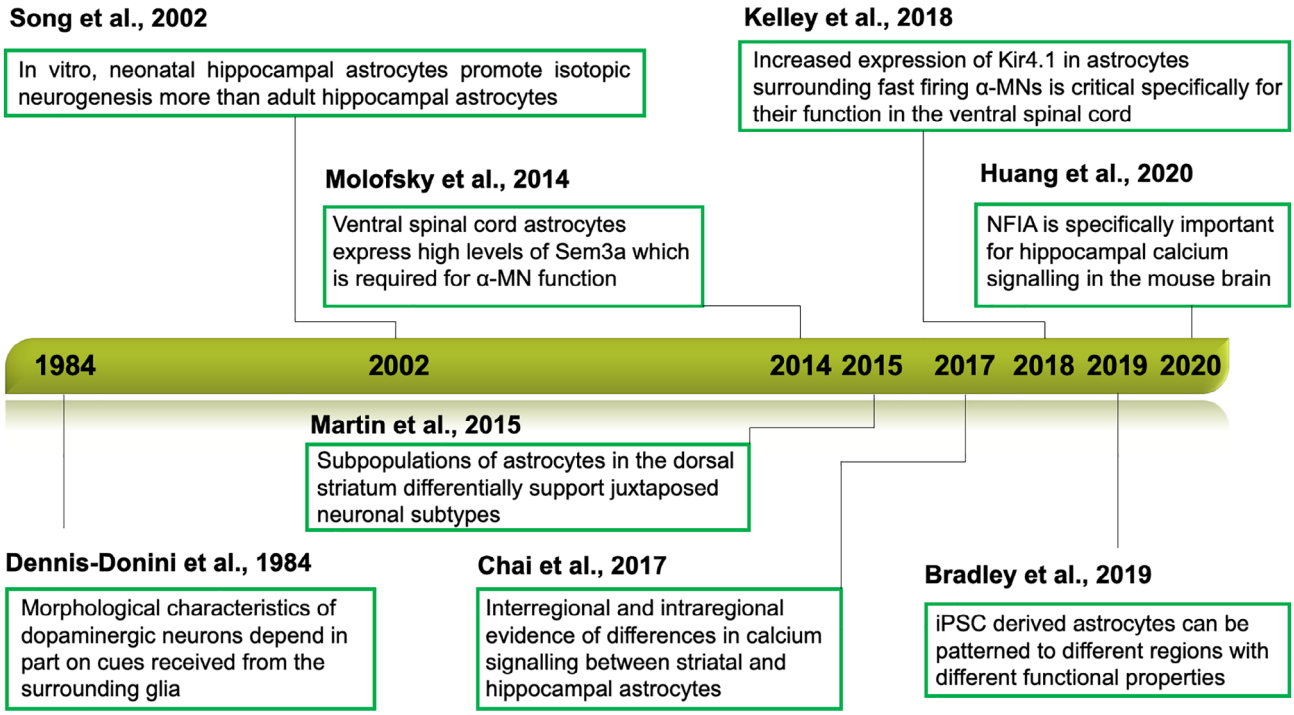

FIGURE 3 Timeline of important studies demonstrating functional heterogeneity of astrocytes. While astrocyte functional heterogeneity was first hinted at over 30 years ago, recent studies have cemented the concept of regionally different functional capabilities of astrocytes

cerebellum or Muller cells in the retina (Emsley & Macklis, 2006). More recently, regionally encoded differences in astrocytes have been extensively described at a molecular level (Bachoo et al., 2004; Doyle et al., 2008; John Lin et al., 2017; Molofsky et al., 2014; Morel et al., 2017) including analysis at the single cell level (Batiuk et al., 2020; Bayraktar et al., 2020; Zeisel et al., 2018) and have furthermore suggested that these molecular differences translate into altered astrocytic function.

An important study tested whether astrocytes allocated to different spinal cord domains are uniquely specialized to support region-specific neuronal circuits. Purified astrocytes from microdissected dorsal and ventral postnatal spinal cords were first examined for regionally-determined molecular differences (Molofsky et al., 2014). Sem3a was most highly expressed in ventral astrocytes and progressively decreased in expression toward the dorsal domain. Conditional ablation of astrocytic Sema3a resulted in the failure of alpha motor neurons (α-MNs) to maintain their axon initial segment toward the ventral root at early postnatal stages and later resulted in specific α-MN death. Additionally, reduced frequency of both α-MNs spontaneous excitatory postsynaptic currents (sEPSCs) and increased spontaneous inhibitory postsynaptic currents (sIPSCs) was observed. Cumulatively, these findings demonstrate that ventral astrocytes are specialized to maintain sensorimotor circuit integrity in α-MNs.

Astrocytes within specific brain regions have also demonstrated significant functional heterogeneity. Hypothalamic astrocytes have been associated with region-specific roles in both insulin signaling (García-Cáceres et al., 2016) and leptin-dependent feeding behaviors (Kim et al., 2014). The transcription factor NFIA, which is important in astrogliogenesis throughout the CNS, has also recently been implicated in region-specific functional effects in the hippocampus (Huang et al., 2020). Astrocyte specific knockout of NFIA caused larger changes in gene expression and significant calcium signaling deficits in hippocampal astrocytes but not in astrocytes in the cortex, olfactory bulb, or brainstem of rodent models.

Recent in vitro data from a study using human induced pluripotent stem cell (iPSC) derived astrocytes further demonstrate regionally encoded functional heterogeneity of astrocytes (Bradley et al., 2019). iPSCs that were patterned to the ventral or dorsal anterior forebrain or spinal cord before being differentiated into astrocytes displayed different electrophysiological properties and optimally supported neuronal outgrowth to neurons patterned to the same region. Furthermore, endothelial cells co-cultured with astrocytes from different regions displayed differences in trans-endothelial electrical resistance, a measure of blood brain barrier formation, which may be due to differences in TGF-β2 secretion between astrocytes from different regions.

Heterogeneity in astrocytic calcium signaling provides further evidence for their specialization within neuroglial units. For example, calcium signaling in ventral brainstem astrocytes has been shown to be critical in mediating neuronal control of breathing (Gourine et al., 2010). One study comprehensively addressed structural and functional astrocyte heterogeneity using an adult rodent model. In this study, astrocytes within the neural circuits of the hippocampus and the striatum were compared comprehensively from morphological, molecular, and electrophysiological standpoints (Chai et al., 2017). Hippocampal and striatal astrocytes displayed similar basic membrane

properties and cell number. However, striatal astrocytes relied more heavily on extracellular uptake to maintain basal calcium levels compared to astrocytes from the hippocampus.

Interestingly, evidence of intraregional astrocytic functional heterogeneity was also raised in this study as dorsal versus ventral parts of the striatum exhibited graded expression of specific factors (Chai et al., 2017). This builds on morphological (Oberheim, Goldman, & Nedergaard, 2012) and molecular evidence from recent single cell RNA sequencing (Batiuk et al., 2020; Bayraktar et al., 2020) of intraregional astrocyte heterogeneity. Interactions with local specific neuronal subtypes may be important for the establishment of intraregional heterogeneity of astrocytes. Functional evidence for this phenomenon has been recently described in astrocytes surrounding fast firing α-MNs in the ventral spinal cord (Kelley et al., 2018). The astrocytic expression of potassium channel Kir4.1 was shown to be critical for their electrophysiological properties. Deletion of Kir4.1 specifically in astrocytes resulted in a loss of size and electrophysiological function in fast firing α-MNs, but not other motor neuron subtypes, adding further evidence for the existence of astrocytes that are specialized to specific neuronal subtypes. Subpopulations of astrocytes from different brain regions have also been reported to differ in their electrophysiological properties (Martín, Bajo-Grañeras, Moratalla, Perea, & Araque, 2015; Morel et al., 2019) and in their ability to support synaptogenesis (John Lin et al., 2017), suggesting that intraregional functional heterogeneity occurs throughout the CNS. Taken together, these findings suggest a finely grained functional specialization of astrocytes within the CNS.

4 | THE IMPACT OF AGING ON ASTROCYTE FUNCTIONAL HETEROGENEITY

From previously described literature, the regional identity of astrocytes is reported to be configured early during development and is maintained into adulthood (Tsai et al., 2012). Indeed, most studies detailed above have suggested that heterogeneity of astrocytes is most evident at developmental stages. However, highly significant alterations in astrocytic gene expression occur upon aging, including region-specific changes. A recent study comparing gene expression of multiple regions of old and young human brains, demonstrated that astrocytes undergo far larger changes in gene expression than neurons, including apparent loss of their regional identity (Soreq et al., 2017). Importantly, the most pronounced differences were reported in the hippocampus and substantia nigra, archetypal foci for Alzheimer's and Parkinson's disease respectively (Soreq et al., 2017). Global changes in astrocytic gene expression have also been found across multiple brain regions in mice (Boisvert, Erikson, Shokhirev, & Allen, 2018; Clarke et al., 2018). Aged astrocytes expressed a reactive state, which differed between the hippocampus, striatum, and cortex. Several markers that were upregulated included those that are expressed in a neuroinflammatory A1-like reactive state. An A1 reactive state has previously been associated with both an increase in neurotoxic pro-inflammatory cytokines and a decrease in astrocytic homeostatic functions (Liddelow et al., 2017). These studies suggest that upon aging, astrocytes undergo a reactive transformation and may lose homeostatic functions, which bear potential significance for region-specific vulnerability in neurodegeneration (discussed below).

In the aging brain, it seems that there is a decline in synaptic function and number together with a general reduction in neuronal activity, but without significant decline in neuron or astrocyte number (Samson & Barnes, 2013). Analysis of astrocytic gene expression in aged mice brains revealed a downregulation of genes involved in synapse regulation and an increase in expression of genes involved in synapse elimination across multiple brain regions (Boisvert et al., 2018). This may be compounded by intrinsic differences in astrocyte synaptogenic factors that have been reported between different regions at postnatal ages (Buosi, Matias, Araujo, Batista, & Gomes, 2018). Alterations in cytokine production and increased oxidative stress may also result in detrimental effects on astrocytic functions during aging such as impaired blood brain barrier regulation and metabolic homeostasis of neurons, however the role of astrocyte heterogeneity in these processes is currently unexplored (Palmer & Ousman, 2018). Therefore, aging may cause a neurotoxic reactive state in astrocytes in conjunction with a loss of supportive capabilities affecting several different brain regions. However, the relevance of regional heterogeneity to the aging process on astrocyte function is only beginning to be understood and further functional assessment of aged astrocytes is required to confirm the current molecular evidence for their proposed neurotoxicity or loss of function.

5 | THE IMPLICATIONS OF ASTROCYTE FUNCTIONAL HETEROGENEITY IN NEURODEGENERATION

Ageing is a major risk factor for several neurodegenerative diseases, which are characterized by the loss of specific neuronal populations. It is now well established that astrocytes are involved in the pathologies of several neurodegenerative conditions (Oksanen et al., 2019; Phatnani & Maniatis, 2015). Regionally encoded heterogeneity of astrocytes may affect the mechanisms of neurodegeneration in several ways and is likely to be dependent on the type and chronicity of the stimulus. Loss of specific neuronal populations may be at least in part due to the failure of functional heterogeneity of certain astrocyte populations in areas affected in disease. For example, in amyotrophic lateral sclerosis (ALS) loss of the potassium channel Kir4.1 in astrocytes that specifically surround fast firing α-MNs may explain the high vulnerability of this neuronal subpopulation to degeneration in ALS (Kelley et al., 2018). Developmentally established regional differences in astrocyte populations encoded during development may also confer vulnerability to certain neuronal subpopulations in disease conditions. An example of this comes from a recent study in which astrocytes from the ventral tegmental area, but not the substantia nigra, expressed the neuroprotective molecule GDF15, which may partly explain the vulnerability of neurons in the substantia nigra in Parkinson's disease (Kostuk, Cai, & Iacovitti, 2019).

Developmentally programmed regional differences in glial populations may also affect subsequent changes in reactivity that occur in injury or disease and thus be more or less damaging to juxtaposed neurons. For example, hippocampal and cortical astrocyte cultures underwent reactive gliosis more than cerebellar or spinal cord cultures when incubated with identical concentrations of Alzheimer's disease related protein β-amyloid (Höke, Canning, Malemud, & Silver, 1994). Furthermore, in both the experimental autoimmune encephalitis (EAE) model of multiple sclerosis and the SOD1 mouse model of ALS different gene expression changes that occurred in diseased astrocytes were region-specific (Itoh et al., 2018; Miller, Glatzer, Hsieh, & Rothstein, 2018; Miller, Zhang, Glatzer, & Rothstein, 2017). Astrocyte reactivity may be described as a continuum between neurotoxic A1 and neuroprotective A2 states based on transcriptional profiles (Liddelow et al., 2017; Liddelow & Barres, 2017; Zamanian et al., 2012). However, this may be an oversimplification as recent data suggests that different diseases are likely to elicit specific temporally mediated gene expression signatures that include a subset of both A1 and A2 markers (Diaz-Castro, Gangwani, Yu, Coppola, & Khakh, 2019). The architecture of astrocyte reactivity is therefore highly complex and may be context dependent, with specific effects on the functions of astrocytes from different regions not yet experimentally validated.

6 | MODELING ASTROCYTE HETEROGENEITY IN NEURODEGENERATIVE DISEASES

Since the heterogeneity of astrocytes may be important in several neurodegenerative disorders, disease modeling strategies should reflect this. Isotopic culture methods for co-culture experiments provide a more powerful model system in which to conduct investigations into the pathological signatures of distinct neurodegenerative diseases. Mouse models have been critical in understanding astrocyte regional heterogeneity. However, several differences in rodent and human astrocytes have been reported, in their size and morphology, calcium dynamics and the expression of more than 600 genes (Oberheim et al., 2009; Zhang et al., 2016). Therefore, taking advantage of iPSC technology or transdifferentiation of patient fibroblasts, which are able to retain age-related transcripts, alongside animal models of disease should further inform disease modeling (Mertens et al., 2015; Tyzack, Lakatos, & Patani, 2016).

Human iPSCs can be patterned to different regions of the developing CNS through the sequential application of an ontogeny-recapitulating program of extrinsic cues (Zirra, Wiethoff, & Patani, 2016), enabling the comparison of regionally distinct astrocyte populations from identical lines (Krencik et al., 2011). Recently, human iPSCs have been able to capture regional differences in astrocyte function (Bradley et al., 2019), however this paradigm has not yet been extended to study the effects of astrocyte heterogeneity on disease phenotypes. Identification of markers that are differentially expressed between regionally distinct populations of astrocytes modeling disease phenotypes may provide targets for future therapeutic intervention by promoting specific protective aspects of astrocyte function or limiting harmful neuroinflammatory activation.

7 | CONCLUDING REMARKS

In this article, we have discussed the increasingly recognized importance of regionally allocated functional heterogeneity in astrocytes and its relevance to aging and neurodegenerative diseases. Understanding the molecular and functional heterogeneity of astrocytes and how they are affected in the context of aging and neurodegenerative disorders may provide a more targeted strategy for therapeutic interventions in future.

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ORCID

Rickie Patani https://orcid.org/0000-0002-3825-7675

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