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Human astrocytes are distinct contributors to the complexity of synaptic function

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Abstract

Cellular components of synaptic circuits have been adjusted for increased human brain size, neural cell density, energy consumption and developmental duration. How does the human brain make these accommodations? There is evidence that astrocytes are one of the most divergent neural cell types in primate brain evolution and it is now becoming clear that they have critical roles in controlling synaptic development, function and plasticity. Yet, we still do not know how the precise developmental appearance of these cells and subsequent astrocyte-derived signals modulate diverse neuronal circuit subtypes. Here, we discuss what is currently known about the influence of glial factors on synaptic maturation and focus on unique features of human astrocytes including their potential roles in regenerative and translational medicine. Human astrocyte distinctiveness may be a major contributor to high level neuronal processing of the human brain and act in novel ways during various neuropathies ranging from autism spectrum disorders, viral infection, injury and neurodegenerative conditions.

Keywords

astrocytes; synapses; neurodevelopmental disorders; neuropathologies

Introduction

Integrative brain function is regulated by neural circuits at a fundamental level that requires assembly during development and refinement throughout life. These circuits are made up of multiple interacting cells, including excitatory and inhibitory neuronal subtypes as well as glial cells, and are regulated by a combination of intrinsic and extrinsic signals that vary according to developmental stage, brain region and sensory experience. These circuits control all aspects of cognition, somatosensation, motor skills and are interconnected by long range signaling. Synaptic circuit wiring and specificity relies upon at least two major factors; the onset of remodeling at the presumptive synapse regulated by neuron-intrinsic competency and the presence of extracellular synapse-modulating adhesion molecules (de

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Wit and Ghosh, 2016). A fundamental question in neuroscience is how evolution of these neural circuits has led to the heightened processing capacity that is characteristic of the human brain. One aspect that has been hypothesized to contribute to this competence is the uniqueness of human astrocytes (Oberheim et al., 2006). In the last decades it has become clear that astrocytes are active players in neurotransmission as illustrated by their contribution to synaptic development and function. Though species differences have been observed in neuronal and synaptic characteristics (DeFelipe et al., 2002), there is strong evidence that human astrocytes have been one of the most divergent neural cell types during evolution of the nervous system. Thus, the unique features of human astrocytes must be taken into account when considering strategies to target the human brain for clinical therapeutics.

This review will focus on extracellular signals from astrocytes that are implicated in regulating formation, maturation, refinement and plasticity of synapses. Though astroglial function has not been as well studied as their neuronal counterparts, there has been a barrage of data in the past decade revealing their contributions to extracellular matrix (Dzyubenko et al., 2016; Farhy Tselnicker et al., 2014), production of synaptic signaling molecules (Harada et al., 2015), neuroplasticity (Haydon and Nedergaard, 2015; Sims et al., 2015) and neuroprotection (Garzon et al., 2016; Liu and Chopp, 2015). Here we will not attempt to reiterate these concepts, but rather contemplate how the findings from studies (mainly conducted in rodent experimental models) implicate a possible important role in the distinctiveness of astrocytes in complex human brain function. This concept has relevance for aging and disease as alterations in astrocyte-derived signals may lead to synaptic dysfunction in psychiatric disorders (Elsayed and Magistretti, 2015; Koyama, 2015), neurodevelopmental syndromes (Molofsky et al., 2012; Sloan and Barres, 2014), epilepsy (Robel and Sontheimer, 2015) and neurodegenerative disorders (Ben Haim et al., 2015; Phatnani and Maniatis, 2015; Radford et al., 2015).

As discussed below, human astrocytes have distinct characteristics compared to those in rodents, yet studies of their development and function have been historically hindered due to technological impediments. However, there have been recent breakthroughs in human tissue processing, cell culture methods, gene expression profiling, and human-mouse chimera techniques that now allows for in depth investigations on normal and pathological cellular function (Table 1). Based upon decades of studies revealing the relationship between astrocytes and synapses in animal models, these lessons can now also be applied to human cells to determine whether different genetic and environmental backgrounds play a role in species-specific differences. Importantly, the identification of human astrocyte-derived signals and their downstream mechanisms that modulate synaptic transmission will be a major advance towards drug development in many, if not all, neuropathologies.

Astrocytes glue together synaptic formation and function

The contribution of glia in the development and regulation of synapses is a widely acknowledged principle that has been progressively studied in the last few decades (Ullian et al., 2004). By combining biochemistry, imaging techniques, transgenic mouse models, and cell culture studies the mysteries underlying the mechanisms of these functions are

becoming unraveled. In rodents, astrocytes are known to promote structural formation of excitatory synapses by release of thrombospondins (Christopherson et al., 2005), proteins that interact with alpha2delta-1 (Eroglu et al., 2009) and neuroligin 1 (Xu et al., 2010). Similarly, the release of Hevin mediates excitatory synapse maturation (Kucukdereli et al., 2011) by linking synaptic proteins neurexin-1 alpha and neuroligin-1B (Singh et al., 2016). Further synaptic support is provided by regulating the availability of cholesterol complexed to apolipoprotein E-containing lipoproteins (Mauch et al., 2001) and through enhancing efficacy via TNFalpha signaling (Beattie et al., 2002) as well as SPARC (Jones et al., 2011). Not only structural formation, but also strengthening of functional synapses (through increasing AMPA receptor surface levels and clustering) requires synaptogenic astrocyte-derived factors such as the heparin sulfate proteoglycan glypican (Allen et al., 2012). Glypican 4 interacts at the presynapse with PTP σ (Ko et al., 2015) and trans-synaptically with the postsynaptic protein LRRTM4 (de Wit et al., 2013). Compared to the influence upon excitatory synapses, less is known about the mechanistic action of astrocytes on inhibitory synapses, though glial cells influence inhibitory currents (Kang et al., 1998) and secrete modulatory soluble factors (Hughes et al., 2010), with TGF- β 1 being one molecule identified to increase inhibitory synapses (Diniz et al., 2014). Altogether, as extensively reviewed by others (Chung et al., 2015; Zuchero and Barres, 2015), multiple studies suggest that the extracellular environment, glued together by glia-secreted proteins and modulators, play a major role in synaptic formation and refinement. Yet, whether the same mechanisms are involved in the human brain and at what developmental time points these processes occur is unclear due to current technical limitations. In light of this, we have reported proteomic profiles of human astrocyte conditioned media and have observed the presence of several synaptogenic factors (Krencik et al., 2015).

Glial engulfment of synapses is a postnatal activity-dependent process of elimination that occurs after the majority of synapses have formed (Chung et al., 2015). Using the well-studied mouse retinogeniculate system as a model of synaptic refinement, it has been demonstrated that astrocytes engulf synaptic material at an equal or greater extent than microglia during postnatal development in a MEGF10 and MERTK-dependent manner (Chung et al., 2013). Furthermore, the same study revealed that engulfment of both excitatory and inhibitory synapses takes place in the adult somatosensory cortex. The phagocytic activity of astrocytes appears to be evolutionarily conserved, as *Drosophila* astrocytes have shown to be the primary cells that phagocytose synapses in the pupal stage via the Draper (ortholog of MEGF10) signaling pathway and Crk/Mbc/dCed-12 complex (Tasdemir-Yilmaz and Freeman, 2014). Primary adult human astrocytes in culture also promote synaptogenesis, engulf synapses, and express genes involved in these processes such as SPARCL1 and MERTK, respectively (Zhang et al., 2016). Thus, it will be important to determine to what extent synaptic engulfment by astrocytes takes place in the developing and adult human brain, not only to gain knowledge about neural circuit maintenance and refinement, but also in light of possible disturbances of this process in neurocognitive disorders.

After synaptic networks have been established, astrocytes have a multifaceted influence upon various aspects of the local environment necessary for synaptic stability and maintenance. At the global level, astrocytes contribute to synaptic health by their well-

known roles in generating a homeostatic environment through potassium and glutamate buffering (Cheung et al., 2015), waste elimination (Jessen et al., 2015), energy metabolism (Magistretti and Allaman, 2015) and by providing neurovascular coupling (Nuriya and Hirase, 2016). More locally, glial cells contribute to neurotransmission by influencing extrasynaptic currents (Pal, 2015) and by influencing myelination of axons (Kiray et al., 2016). Furthermore, there is experimental evidence that astrocytes release transmitters to directly regulate neuronal synaptic transmission, commonly termed “gliotransmitters” such as D-serine and ATP, yet the precise mechanisms of how gliotransmitter release is regulated and to what extent they influence synapses is still at the conceptual stage (Araque et al., 2014). The close and dynamic interaction of astrocytes with mature synapses is enabled by protrusions of fine, ramified membrane structures termed perisynaptic astrocyte processes (PAPs), so far almost exclusively studied in rodent astrocytes. These protrusions from the periphery of astrocytes have been difficult to investigate due to their small size and lack of abundant protein markers, though recently they have become more recognized as integral components of the neuronal synapse modulatory machinery (Ghezali et al., 2015). The structural plasticity of PAPs, including the amount of synaptic area that is surrounded by these membranes, is partly regulated by the level of excitatory synapse activity and is associated with an increased stability of spines (Bernardinelli et al., 2014). Within the processes, neuronal activity also induces mitochondria repositioning near synapses (Stephen et al., 2015) and this event is associated with intracellular calcium elevations in PAPs. To what extent local activity regulates calcium transients however remains controversial, as it has been demonstrated that the effect of local activity is strongly regulated by noradrenergic and cholinergic inputs *in vivo* (Bazargani and Attwell, 2016; Paukert et al., 2014). Gap junction protein connexin 30 is one specific protein that has been found to regulate synaptic strength by controlling the extent of glutamate transporter-containing PAP protrusion into the synaptic cleft, thereby adjusting the amount of available glutamate at the synapse (Pannasch et al., 2014). Altogether, due to the close proximity to synapses (Figure 1C), PAPs may underlie a critical structural mechanism for fast communication between astrocytes and neighboring cells as described in the “astroglial cradle” model (Verkhratsky and Nedergaard, 2014). Where and how do astrocytes arise in order to provide these functions? Next, we discuss what is known about their genesis from neural stem cells and the possible implications for human cognitive dysfunction when this developmental process is perturbed.

Astrogenesis is essential for synaptogenesis

The importance of astrocytes is reflected by the temporal association between astrogenesis and synaptogenesis. The majority of functional synapses do not form until astrocytes appear and this can be accounted for by the intrinsic inability of neurons to form numerous synapses as well as the presence of astrocyte-released synaptogenic factors described above that are not produced in high quantity by progenitor cells. Where and how do these synaptogenic astrocytes appear in the nervous system and how is this unique in the human brain? In order to understand the origins and diversity of astrocytes in different regions of the nervous system, a variety of genetic and clonal lineage tracing studies in transgenic mice have been devised. Based on these studies, it is now an established principle that the majority of astrocytes are generated from the same neuroepithelial-derived radial glia (RG)

that also produce neurons earlier in development. RG in the ventricular zone (VZ) of the cortex undergo a neurogenic-to-gliogenic conversion after the majority of neurons are generated and this is controlled by gene expression regulators at the genetic and epigenetic levels as well as environmental cues that activate intracellular signaling cascades (Miller and Gauthier, 2007). Besides this RG origin, there is also evidence that the glial cells are born via symmetric division of existing astrocytes during early postnatal periods in upper layers of the cortex in mice (Ge et al., 2012) and from gliogenic progenitor pools in the subventricular zone (SVZ) niche. Progenitors in this niche have been observed to contribute to the generation of new astrocytes during early adulthood in response to injury (Benner et al., 2013).

One outstanding question is how the morphological and functional diversity of astrocytes is established during development (Bayraktar et al., 2015). Various lineage tracing experiments in mice have indicated that different subtypes of cortical astrocytes such as those in layer 1, grey matter protoplasmic- and also white matter fibrous-astrocytes originate from different progenitor pools (Tabata, 2015). However, to what extent these populations display regional diversity and how functional diversity is established is only starting to be understood. Astrocytes appear to remain in a restricted region tangential to their site of embryonic origin (Tsai et al., 2012) and this intrinsic regional-specification has been shown to have consequence on motor and sensory circuit maintenance in the spinal cord (Molofsky et al., 2014). Their identity does not appear to be solely determined by embryonic specification, however, since local signals from adult neurons in the cerebellum also affect expression and function of neighboring astrocytes in the form of sonic hedgehog protein (Farmer et al., 2016).

In the human brain, cortical protoplasmic astrocytes first appear around 20 weeks of gestational age and their density continues to increase through birth (Roessmann and Gambetti, 1986). How do these developing cells compare to those in rodent? By examining RG transcriptional signatures of human (gestation week 14.5) to that against mouse (embryonic day 14.5) it was found that RG gene expression is broadly conserved, yet the PGDF-PDGFR β signaling pathway was identified as uniquely important for human cortical RG proliferation (Lui et al., 2014). In addition, a particular aspect of cortical development is that humans and other gyrencephalic mammals contain an additional zone of proliferation distinct from the SVZ, rarely observed in mouse brain and termed the outer SVZ (OSVZ), that is thought to account for an expanded volume, density and long cortical developmental period (De Juan Romero and Borrell, 2015). Progenitor cells that reside in the OSVZ, termed outer radial glia (oRG), can be distinguished from ventral radial glia by markers including HOPX as well as extracellular matrix related markers PTPRZ1 and TNC. oRG are readily distinguishable from astrocytes by their lack of marker expression that appear later in development including AQP4, IL33 and ALDOC (Pollen et al., 2015). Immunoanalysis and live imaging studies of oRGs have revealed that these cells display a unique and dynamic morphology and behavior during cell division, suggesting an important role in orchestrating proper gyrated cortical structure (Fietz et al., 2010; Gertz et al., 2014; LaMonica et al., 2013). Human neural development is now commonly investigated by *in vitro* studies utilizing human pluripotent stem cells (hPSCs) (Krencik and Zhang, 2006). Several aspects of oRGs have been recapitulated during hPSC differentiation in three-dimensional cultures

(Kadoshima et al., 2013; Otani et al., 2016). Remarkably, this developmental timing of RG-to-astrocytes that has been measured *in vivo* is mirrored by hPSC differentiation, providing evidence of a cell-intrinsic developmental program (Krencik and Zhang, 2011).

What happens when the gliogenic switch or timing of astrocyte appearance is disrupted? Abnormalities before and during astrogenesis have been implicated to contribute to phenotypes described in human neurodevelopmental syndromes including those displaying defective neural networks (Sloan and Barres, 2014) and brain malformations such as lissencephaly (Budday et al., 2015). By applying mosaic analysis with double markers (MADM) technology to mouse models of lissencephaly via *Lis1* gene targeting, neural migration and subsequent generation of neurons and astrocytes was found to be severely disrupted in several areas of the brain (Hippenmeyer et al., 2010) supporting the idea that a decrease in astrocyte number, similar to neurons, may be linked to declined cognition. Astrocyte dysfunction has also been implicated in neurodevelopmental disorders sometimes associated with microcephaly including Down's syndrome, Rett syndrome and Fragile X syndrome (Molofsky et al., 2012). In support of this, several autism-spectrum disorder-linked genes identified by genome wide association studies are expressed, or in some cases enriched, in purified mouse astrocytes (Sloan and Barres, 2014). Likewise, by using human gene expression and cell culture analysis, the disruption in prenatal brain morphology and microcephaly observed during human Zika virus infection has recently been implicated to be caused by disturbances in the properties of RG and in their progeny (Nowakowski et al., 2016; Qian et al., 2016; Tang et al., 2016; Wu et al., 2016). Though the mechanistic consequences of Zika virus infection are unclear, activation of the TLR3 pathway has been recently identified as one contributing mechanism that could be involved in the regulation of various downstream disease-related cellular responses (Dang et al., 2016). Conversely, in one large family of disorders linked to macrocephaly, termed RASopathies (Rauen, 2013), accelerated astrocyte development, and consequently, altered timing of synaptic development, have been observed (Krencik and Ullian, 2013; Krencik et al., 2015). These powerful astrobiological influences on brain function argues the need to integrate astrocytes as essential components of the excitatory-inhibitory synaptic homeostasis imbalance that is proposed as an underlying causation of numerous cognitive and social impairments including those involving human autism spectrum disorders (Mullins et al., 2016).

Do human astrocytes belong to an exclusive club?

The uniqueness of human astrocytes is not a new concept. Even when human astrocytes were first carefully described in the drawings by pioneering scientists over a century ago, the complexity of the glial population in the human brain could already be appreciated (Kettenmann and Verkhratsky, 2008). We are now just beginning to understand the heterogeneity of human astrocytes and the divergence of these cells among species. Compared to their rodent counterparts, human proteoplasmic and fibrous astrocytes are larger, have a more complex morphology and propagate calcium waves faster (Oberheim et al., 2006; Oberheim et al., 2009). Early evidence for a uniquely primate subtype came from analysis of brain tissue revealing a morphologically unique interlaminar type (Figure 1A) characterized by a long radial process from cortical layer 1 that extends from layer 1 to layers III/IV; a feature that becomes more apparent during adulthood and is also observed to

a lesser extent in the non-human primate brain (Colombo et al., 1997). Several hypotheses were put forth about the functional relevance of these elongated processes ranging from a mechanism for spatial segregation limiting the diffusion of molecules, a system to improve redistribution of potassium and an infrastructure for long-range signal transmission (Colombo and Reisin, 2004; Reisin and Colombo, 2002). In addition to the interlaminar astrocytes, the primate brain is also populated by another type of projecting astrocyte localized in layer V/VI of the cortex termed varicose projecting astrocytes. Long process-bearing human astrocytes in the cortex and hippocampus can be identified by the cell surface protein CD44 and markers such as SPARC, CRYAB, S100 β , GFAP and AQP4. Besides the additional subtypes of astrocytes, the organization of astrocytic networks is also different in the human brain compared to other species. In *Drosophila* (Stork et al., 2014), rodent (Bushong et al., 2002; Ogata and Kosaka, 2002) and, to a much lesser extent, ferret (Lopez-Hidalgo et al., 2016), the organization of proteoplasmic astrocytes is characterized by ‘tiling’ of minimal-overlapping spatial domains. Although tiling also occurs in human protoplasmic astrocytes, the spatial domains of these cells are more overlapping and the boundaries are not respected by processes from varicose and interlaminar projecting astrocytes (Oberheim et al., 2009; Sosunov et al., 2014). Given the uniqueness of human astrocytes, a substantial step forward will be to experimentally examine what the functional consequences are of these morphological distinctions. Progress in this field has been impeded due to the difficulty in collecting and processing human tissue. Recently, a method has been devised to process human brain tissue after long term storage while maintaining structural organization, allowing the study of three-dimensional anatomy of astrocytes in healthy and diseased tissue (Bouvier et al., 2016). Furthermore, methods to acutely purify astrocytes from human tissue have been improved using an immunopanning strategy with the cell surface protein HepaCAM and this led to the observation that human cellular uniqueness can be preserved in culture (Zhang et al., 2016). Successful attempts to replicate the complex human astrocyte morphology in cell culture have been minimal, but some progress has been made in recapitulating the stellate morphology by optimizing plating substrates (Levy et al., 2014; Placone et al., 2015).

Emerging bioinformatic approaches are also revealing human astrocyte uniqueness at the level of gene expression and this suggests important functional pathways previously obscured. Neuron-enriched genes were reported to be well preserved in evolution, in contrast, glial-enriched genes have been shown to be evolutionarily divergent (Hawrylycz et al., 2015). This suggests that selective pressures influenced human astrocyte evolution and therefore the comparison of species differences may bring us one step closer to understanding the unique roles of astrocytes in human brain function (Freeman and Rowitch, 2013). Direct comparison of transcriptomics of acutely isolated human and mouse astrocytes led to the identification of select species-enriched gene expression differences and may bring to light the functional relevance of several different pathways such as those involved in Wnt signaling (WIF1) and calcium signaling (Ryr3) (Zhang et al., 2016). In addition to inter-species differences in gene expression, it will be important to understand whether human astrocytes in different regions of the brain have unique sets of gene signatures.

What are the consequences of the human-associated morphological, functional and transcriptional phenotypes upon interactions with other cell types within a synaptic circuit

and on the integration of astrocytes in the large scale neuronal network? The increase in size and complexity will undoubtedly have an effect on the synaptic coverage and potentially on the function of a single astrocyte; thus affecting networks of neurons and their circuit integration (Heller and Rusakov, 2015; Hu et al., 2016). This indicates that human astrocytes act upon synaptic function in exceptional ways that cannot be examined solely using non-primate experimental models. Though, a majority of astrocyte functions are indeed conserved between multiple species and the use of model organisms will be indispensable to further reveal unknown roles. Devising experimental strategies that allow us to study the effect of human astrocytes on their micro- and macro-environment will be essential to understand specific aspects of human brain function and dysfunction in motor, sensory, cognition and social processing. In the next section, we highlight some emerging technologies that make use of such systems and hold the potential for advancing our knowledge.

Emerging tools elucidate cellular interactions

Successful sustained engraftment of human astrocytes into the rodent brain has been a major advance to interrogate human astrocyte function in complex neural networks (Goldman et al., 2015). hPSC-derived astrocytes retain their primate-specific complex morphology and regional specific characteristics in the presence of the mouse brain environment (Krencik et al., 2011). Functionally, engrafted human astrocytes present faster calcium wave propagations, confer an increased long term potentiation of neighboring hippocampal neurons and promote an enhancement of learning in several behavior tasks compared to endogenous cells and non-engrafted controls (Han et al., 2013). The transplantation of donor cells into a non-native system has received much attention due to the possible application as therapy, but until the many obstacles that are faced today are overcome, this system may be best applied as a research tool for neurodegeneration and regeneration (Goldman, 2016) as recently demonstrated with Huntington disease chimeras (Benraiss et al., 2016). This type of strategy has also been used as a model for amyotrophic lateral sclerosis and engraftment of human astrocytes expressing mutant SOD1 into a mouse spinal cord resulted in loss of motor neurons and motor function (Chen et al., 2015). Transplantation of glutamate transporter-overexpressing astrocytes, on the other hand, was shown to have a beneficial effect on spinal cord injury recovery by prolonging diaphragm innervation, demonstrating that cellular engraftment can also be used as tool to study regeneration (Li et al., 2015). The maturation of the engrafted cells appears to be influenced by environmental cues, as fetal human neural progenitor cells have more efficient astrocyte differentiation in aged versus young rats (Das et al., 2016). All things considered, this field of research is in its infancy and it will be crucial to better understand how engrafted cells act and interact with host cells in various regions of the brain and disease states, as well as track the subtype identity of the transplanted astrocytes. Progenitors specified to generate regional specific astrocytes may ultimately yield the most suitable cell type for transplantation into specific regions of the nervous system to support neuronal subtype function.

Another arising technology to generate a more native environment with human cells is by culturing them as three dimensional spheres, commonly termed organoids or mini-brains, to reproduce nervous system structure and extracellular signaling. Single cell RNA-sequencing

studies revealed that neural progenitor cells within organoids give rise to RG and neurons in discrete cortical layers by similar gene expression network patterns as within the human fetal brain (Camp et al., 2015). Organoid cultures have also been used to examine disease related phenotypes as models of microcephaly (Lancaster et al., 2013), Alzheimer's disease (Choi et al., 2014), autism spectrum disorders (Mariani et al., 2015) and Zika virus infection (Dang et al., 2016; Nowakowski et al., 2016; Qian et al., 2016). As expected, astrocytes appear in organoids after long term culture at time points similar to *in vivo* development and this timing corresponds with mature synaptic function (Pasca et al., 2015; Qian et al., 2016). Investigations into the influence of astrocyte function upon synapse physiology in these and future models will undoubtedly be a major addition to the scientific tool kit.

Speculations on high level processing

The expanded appreciation of astrocyte involvement in synaptic circuit processing together with the observed unique traits of the cells in humans has raised the question whether astrocytes could be a potential cellular substrate for human cognition and consciousness. Though our knowledge to date about complex cognition and consciousness is limited at the cellular level, several hypotheses have been put forward for how human astrocyte complexity may affect high level processing. With its numerous multi-directional processes (Figure 1B), a single human protoplasmic astrocyte covers a large domain in the cortex and has thereby the calculated potential to signal to ~270,000 to 2 million synapses (Oberheim et al., 2009). In addition to the interaction with neurons, astrocytes can also communicate with neighboring networks of glial cells via gap junctions and may form a panglial syncytium across large regions of the nervous system (Theis et al., 2005). It has been hypothesized that these extensive and far reaching networks of human astrocytes could be a major player in consciousness (Robertson, 2013) and time-dependent memory formation (Zorec et al., 2015). Similarly, the calcium wave dynamics used by astrocytes for intracellular- and network-communication has been proposed to be an underlying mechanism for information integration and global brain synchronization (Pereira and Furlan, 2009; Pereira and Furlan, 2010). Expansion of the size and number of astrogial processes, a phenomenon observed across multiple models of neurodevelopmental syndromes including autism spectrum disorders, has been hypothesized to lead to hyperconnectivity or “mega-domains” that could underlie savant abilities (Mitterauer, 2013b) and increased intuition when combined with a highly developed memory capability (Mitterauer, 2013a). This may not be a farfetched notion considering the fine synaptic tuning possibilities at the dynamic PAPs (Ghezali et al., 2015). We have recently found that human astrocytes secrete multiple components of perineuronal net proteins that are known to enwrap subpopulations of interneurons in the cortex and stabilize synapses (Krencik et al., 2015), and a compelling hypothesis has been proposed that these structures may be another mechanism for very long term memories (Tsien, 2013).

The roles of human astrocytes in cognition and consciousness described above are highly speculative, yet innovative *in vivo* strategies in rodents have arisen in the past decade that are making strides to understand how astrocyte incorporation into neuronal networks may influence integrative brain function and behaviors, as extensively reviewed elsewhere (Oliveira et al., 2015). One exciting recent strategy that utilized the combination of

optogenetic-based stimulation, intracellular calcium imaging, extracellular glutamate imaging and measurements of local field potential activity, has uncovered a relationship between cortical astrocyte activity and the shift to slow oscillation states; thus possibly linking neural circuit modulation with the memory consolidation that occurs during slow-wave sleep (Poskanzer and Yuste, 2016). In another recent optogenetic-based study in the hippocampus, astrocytes were discovered to act as intermediaries between the memory-related signaling of cholinergic neurons to inhibitory interneurons (Pabst et al., 2016). Despite progress in the field, thus far there is no consensus on the exact role of astrocytes in the dynamic function of neuronal networks. A better understanding about this interaction in normal and disease states may lead to novel strategies to alleviate cognitive dysfunction (Dallerac and Rouach, 2016).

Conclusions

In the last couple decades, there has been an explosion of data at the molecular, cellular and functional level implicating a crucial role of human astrocytes upon synaptic function. Despite our increasing knowledge, several questions remain unanswered and need to be uncovered. For example, can over- or under-expression of a single gene in human astrocytes transform aspects of human cellular properties into those similar to a rodent, and vice versa? Are there ongoing evolutionary pressures that increase fitness at the cellular or circuit level, resulting in astrocyte diversification? In line with this, it has been proposed that glia have evolved to provide homeostatic maintenance necessary for thicker brains and the extended life span of humans (Verkhratsky and Nedergaard, 2016). Studying the function at the synaptic circuit level is complicated by several factors, including the diversity within astrocyte subtypes, the numerous pre- and post-synaptic types surrounded by astrocyte membranes that are all integrating onto a single neuron, and the contribution of factors originating from the vasculature and extracellular matrix (Figure 1). With the recently developed experimental strategies to tease apart unique aspects of these human cells, the specific mechanisms that are involved in all these processes may be discovered in the near future. Until these improved technologies are optimized and more are devised, the role of astrocytes in high level integration of multiple cortical circuits across the brain may be best understood when applied to computational models for artificial neural networks (Soleimani et al., 2015) or artificial intelligence programs. Given the newfound uniqueness and diversity of this cell type, one can imagine how the complexity of human astrocytes may have greatly increased the likelihood of sparking high level cognitive processing.

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Highlights

Human astrocytes may be major contributors to high level circuit processing.

Identification of synaptic transmission modulators from astrocytes will be a major advance towards drug development.

Emerging technologies to understand astrocyte function upon synapse physiology are discussed.

Figure 1.

Simplified illustration of astrocyte-synapse interactions in the human cortex. A) Interlaminar astrocytes (orange) in layer 1 project into multiple layers of the human cortex though the functional consequence remains unknown. B) Large and complex protoplasmic astrocytes (green) signal between the vasculature (red) and synaptic space of possibly millions of synapses per astrocyte domain. C) Synaptic formation (inhibitory neurons in blue, excitatory neuron in yellow) and transmission is dynamically regulated by closely apposed perisynaptic processes.

Table 1
Select list of recent innovations in human astrocyte research

Reference	Description
Colombo J.A. <i>et al.</i> , 1997	Postnatal interlaminar processes of astrocytes detailed in human brain
Oberheim N.A. <i>et al.</i> , 2006	Human astrocyte morphological diversity and function distinguished from that of rodent
Krencik R. <i>et al.</i> , 2011	Human astrocytes from pluripotent stem cells characterized and engrafted into mice
Han X. <i>et al.</i> , 2013	Human astrocyte chimeric mice generated and analyzed
Hawrylycz M. <i>et al.</i> , 2015	Bioinformatics approach revealed human glial divergence in evolution
Zhang Y. <i>et al.</i> , 2016	Acutely purified and profiled primary human astrocytes in healthy and diseased states