Stem cell-derived astrocytes: Are they physiologically credible?

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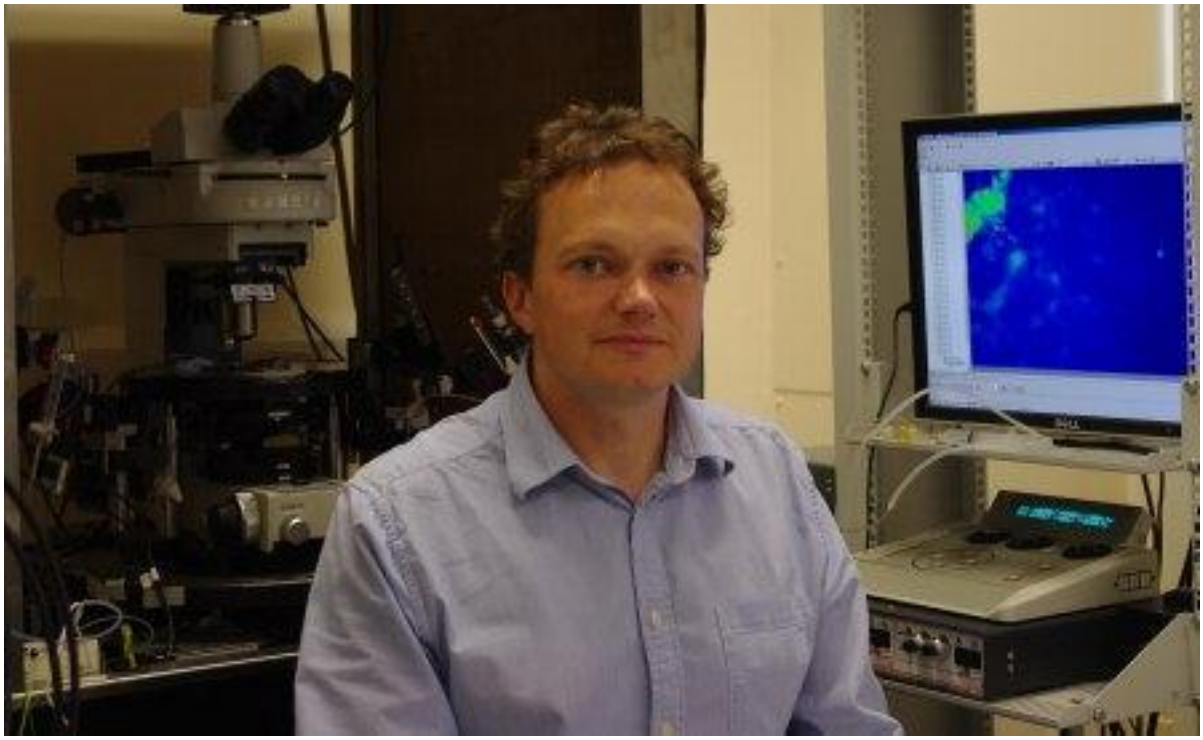

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Abstract

Astrocytes are now increasingly acknowledged as having fundamental and sophisticated roles in brain function and dysfunction. Unravelling the complex mechanisms that underlie human brain astrocyte-neuron interactions is therefore an essential step on the way to understanding how the brain operates. Insights into astrocyte function to date, have almost exclusively been derived from studies conducted using murine or rodent models. Whilst these have led to significant discoveries, preliminary work with human astrocytes has revealed a hitherto

unknown range of astrocyte types with potentially greater functional complexity and increased neuronal interaction with respect to animal astrocytes. It is becoming apparent, therefore, that many important functions of astrocytes will only be discovered by direct physiological interrogation of human astrocytes. Recent advancements in the field of stem cell biology have provided a source of human based models. These will provide a platform to facilitate our understanding of normal astrocyte functions as well as their role in CNS pathology. A number of recent studies have demonstrated that stem cell derived astrocytes exhibit a range of properties, suggesting that they may be functionally equivalent to their in vivo counterparts. Further validation against in vivo models will ultimately confirm the future utility of these stem-cell based approaches in fulfilling the need for human-based cellular models for basic and clinical research. In this review we discuss the roles of astrocytes in the brain and highlight the extent to which human stem cell derived astrocytes have demonstrated functional activities that are equivalent to that observed *in vivo*.

Abbreviations CNS, Central Nervous system; ESC, Embryonic stem cell; iPSC, Induced pluripotent stem cell; EC, embryocarcinoma; Ca^{2+}, Calcium; ATP, Adenosine triphosphate; IP3, Inositol trisphosphate; GLAST, Glutamate/aspartate transporter; GLT-1, Glutamate transporter; ROS, Reactive oxygen species; GSH, Glutathione; GSSG, Glutathione disulphide; GCL, Glutamate cysteine ligase; GSR, GSSG reductase; Nrf2, Nuclear factor erythroid 2; ARE, Antioxidant response element; TCA, Tricarboxylic acid cycle; MCT, Monocarboxylate transporter; GFAP, Glial fibrillary protein.

Introduction

The term ‘glial cell’ refers to a heterogeneous group of various cell types including oligodendrocytes, microglial cells and astrocytes. Astrocytic cells have long been viewed as simple homogeneous cells that carry out supportive housekeeping roles

throughout the brain. However, astrocytes represent a complex and functionally diverse population of cells (Khakh and Sofroniew, 2015) which are now recognised to be intimately involved with neuronal signalling, forming with neurons what is now termed the 'tripartite synapse' (Volterra and Meldolesi, 2005). Together with basic housekeeping activities, astrocytes play key roles in the development and function of neuronal circuitry, as well as CNS-responses to disease states (Barres, 2008). Analysis of the human brain has estimated on average a maximal glial/neuron ratio of 0.99. However, this ratio is very different across brain regions such as cerebellum (0.23), and the grey matter of the cerebral cortex (1.48) when compared with the rest of the brain (11.35) (Herculano-Houzel, 2014).

The physiological roles and properties of astrocytes are now under greater scrutiny and a number of in vivo studies have emphasised the emerging role of astrocytes in previously uncharted processes, which involve executive CNS functional capability, such as information processing and behaviour (Laming et al., 2000). However, key questions regarding the contribution astrocytes make to more basic aspects of neuronal function, such as network activity and disease modulation still remain unexplored.

Mechanistic studies of functional astrocytic-neuron interactions that rely upon imaging and physiological methods, have largely been carried out using in vivo models or ex vivo brain slices or primary rodent cultures. Whilst these models have provided great insight into the complex and diverse roles of astrocytes, some studies have highlighted key differences between human and rodent astrocytes. Oberheim et al (2009) demonstrated that human astrocytes are 2.6 fold larger and extend 10-fold more primary processes, and therefore cover more synapses than mouse astrocytes. In addition, human and ape brains contain astrocyte subtypes that do not exist in the rodent brain (Oberheim et al., 2009). Furthermore, Han et al (2013) observed that engraftment of human astrocytes into mouse brain early in development, enhanced LTP and learning in these human glial chimeric mice. Such findings indicate significant and important roles for human astrocytes and that human

astrocytes are likely to exceed the capabilities of rodent cells. This emphasises the need to develop human models that can explore the most advanced features of astrocyte function. This human-based route will facilitate a more complete understanding of the contribution of astrocytes towards human brain physiology and pathophysiology.

The development of human CNS models has been beset by historic difficulties, such as obtaining significant quantities of viable adult human tissue, as well as ethical concerns regarding the use of (more plentiful) foetal tissue. However, recent advances in stem cell biology have provided a unique opportunity to study the human CNS cell systems in the laboratory. Whilst there is great interest in the use of these cells in regenerative therapies and for modelling human disease, it is essential in terms of model credibility, to determine the functionally of these cells in comparison with their *in vivo* counterparts. Whilst stem cell derived neuronal cells derived from human sources have received a large amount of interest, the potential for forming and studying astrocytic cells and their function, has been largely underexploited.

Roles of astrocytes

In this review we will summarise the diverse roles of astrocytes and discuss the use of stem cells to study these roles in vitro. Numerous studies have now demonstrated significant correlation between the functional characteristics of stem cell derived astrocytes and primary cells. Here we discuss the studies that have demonstrated the functional characteristics of stem cell derived astrocytes in the areas of development, glutamatergic transmission, gliotransmission, oxidative stress, metabolism and disease (Fig. 1).

Role of astrocytes in CNS development

During mammalian CNS development, neural precursor cells differentiate in specific waves, firstly generating neurons followed by astrocytes (Freeman, 2010). This order of development continues during postnatal development (Bandeira et al., 2009).

Astrocytes associate with multiple synapses and coordinate the development of neuronal networks. Indeed, astrocytes are crucial to the development of functional synapses using both secreted (Clarke and Barres, 2013) and contact mediated signals (Barker et al., 2008, Hama et al., 2004, Elmariah et al., 2005). In addition, the maturation of synaptic events *in vitro* is also enhanced by the presence of astrocytes (Johnson et al., 2007, Tang et al., 2013, Hartley et al., 1999). The patterning of neural and astrocytic cells derived from stem cells *in vitro* also occurs in a temporal manner and has been demonstrated in a range of human foetal (Lee et al., 1993, Caldwell et al., 2001) and stem cell types, including embryonic stem cells (ESC) (Krencik and Zhang, 2011), induced pluripotent stem cells (iPSC) (Shaltouki et al., 2013, Roybon et al., 2013) and embryocarcinoma cells (EC) (Bani-Yaghoub et al., 1999).

Synaptic modulation

A key feature of neurons is their ability to communicate with one another and transmit information via synaptic transmission (Carmignoto, 2000). A sophisticated mechanism of bidirectional signalling exists between neurons and astrocytes that coordinates this functional relationship, indicating an important role for astrocytes in the normal functioning of nervous system (LoPachin and Aschner, 1993, Verderio and Matteoli, 2001, Araque et al., 2014).

Astrocytic processes encapsulate numerous synapses in the CNS and are able to modulate synaptic activity (Carmignoto, 2000). Neurotransmitters released at the synapse can activate receptors on astrocytes, inducing sustained cytosolic calcium (Ca^{2+}) elevations or periodic oscillatory activity, which propagates within and between astrocytes (Carmignoto, 2000). Ca^{2+} elevations in astrocytes cause glutamate release from the same cells, which generates a positive feedback stimulus to neurons that modulates neuronal excitability and synaptic transmission enabling astrocytes to integrate extracellular signals and exchange information (Fields and Stevens-Graham, 2002, Perea and Araque, 2002). In addition, astrocytes can also respond to gliotransmitters such as glutamate and ATP that can have paracrine

effects on neighbouring astrocytes and alter neurotransmission (Zhang and Haydon, 2005). Astrocytes also display intrinsic Ca^{2+} oscillations that are not driven by neuronal activity. These oscillations can display regular pacemaker patterns, although the precise role of these patterns is unclear (Parri et al., 2001, Parri and Crunelli, 2001).

We have previously shown that EC derived astrocytes sense neuronal activity and respond to synaptically released neurotransmitters (Hill et al., 2012, Tarczyluk et al., 2013). We have also demonstrated that these astrocytes are able to propagate signals throughout the astrocytic syncytium (Hill et al., 2012). Following activation of G protein coupled receptors the second messenger molecule inositol trisphosphate (IP3) initiates intracellular Ca^{2+} release that is transferred to neighbouring astrocytes through gap junctions. In addition, the release of the gliotransmitter ATP activates purinergic receptors on adjacent cells, thus enhancing the propagation of a resultant calcium wave (Simard and Nedergaard, 2004). Mechanical stimulation of EC and ES derived astrocytes initiate calcium elevations in the stimulated cell that are propagated through the astrocytic syncytium by sequential recruitment of adjacent astrocytes (Hill et al., 2012, Roybon et al., 2013). In EC-derived astrocytes calcium wave propagation was also found to be dependent upon both gap junctions and purinergic signalling, demonstrating gliotransmission in EC derived astrocytes (Hill et al., 2012). These astrocytes also displayed rhythmic calcium oscillations in a manner previously observed in rat astrocytes (Parri and Crunelli, 2001). Whilst the role of this activity is unknown in CNS function, such outputs may have a significant role within neuronal network activity.

Control of synaptic activity

Astrocytes are pivotal in the maintenance of synaptic transmission. The excitatory neurotransmitter glutamate is synthesised in glutamatergic neurons and then accumulated into synaptic vesicles. In response to neuronal stimulation, glutamate is released into the synaptic cleft by calcium dependent exocytosis of synaptic vesicles, producing a stimulus in an adjacent neuron. Glutamate is then deactivated primarily

by transport into surrounding astrocytic processes by a sodium-dependent uptake system involving astrocytic excitatory amino acid transporters (EAAT) such as the glutamate/aspartate transporter (GLAST) and glutamate transporter 1 (GLT-1) (Danbolt, 2001).

Despite its role as a neurotransmitter, glutamate acts as a potent excitotoxin when present at high concentrations at glutamatergic synapses, resulting in excitotoxicity. The over-stimulation of excitatory amino acid receptors by glutamate results in increased levels of cytosolic Ca^{2+}, and the subsequent activation of calcium dependent enzymes including proteases, lipases and nucleases (Garcia and Massieu, 2003), as well as the production of ROS; this is followed by mitochondrial dysfunction leading to necrosis or delayed apoptosis (Almaas et al., 2002). Astrocytic processes closely encapsulate synapses and under normal physiological conditions EAATs reduce the extracellular glutamate concentrations to low nM (e.g. 25nM) levels (Herman and Jahr, 2007). Thus, glutamate transport into astrocytes plays a crucial role in modulating efficient synaptic transmission whilst preventing excitotoxicity (Tzingounis and Wadiche, 2007).

The importance of astrocytes in the maintenance of glutamate concentrations in culture is highlighted by the issue of excitotoxicity. Stem cell-derived neurons have previously been used to study excitotoxicity and demonstrate increased sensitivity to glutamate during differentiation (Munir et al., 1995, Hanko et al., 2006, Gupta et al., 2013). The ability to generate functional astrocytes from stem cells is essential to study normal astrocyte function. Numerous studies have identified the expression of the glutamate transporters GLT-1 and GLAST in stem cell derived astrocytes (Tarczyluk et al., 2013, Shaltouki et al., 2013, Roybon et al., 2013). These proteins are involved in maintaining physiological extracellular glutamate concentrations. Indeed, functional astrocytes derived from EC, iPSC and ESC have demonstrated efficient sodium-dependent glutamate uptake (Shaltouki et al., 2013, Roybon et al., 2013, Serio et al., 2013, Sandhu et al., 2003) and display the functional characteristics of primary astrocytes. Such observations demonstrate the potential

use of these cells as a platform for studying CNS dysfunction *in vitro* as well as the neuroprotective effects of astrocytes in co-culture.

Astrocytic maintenance of cellular glutathione levels

Dringen et al. (1999a) have previously demonstrated that the detoxification of peroxide by neurons is less efficient than that of astrocytes. In addition, astrocytes are able to protect neurons against oxidative stress, through the supply of glutathione (Desagher et al., 1996). They are also able to non-enzymatically scavenge extracellular hydrogen peroxide via the release of pyruvate (Desagher et al., 1997, Wang and Cynader, 2001). Glutathione is present in high concentrations (1-3 mM) in the human brain (Iwata-Ichikawa et al., 1999) and the enzymes for its synthesis, catalysis, interconversion of GSH and GSSG and formation of GSH S-conjugates are all present in the brain (Makar et al., 1994). As with other tissues subject to oxidative stress, glutathione capacity is efficiently maintained by homeostatic means, such that GSSG levels are only approximately 1% of available thiol levels during normal (non-oxidative stress) conditions (Sagara et al., 1996).

Studies suggest that GSH in the CNS is more concentrated in astrocytes, and that astrocytes also possess higher levels of GSH synthesising machinery and exporting capacity, which protects surrounding neurons against oxidative insults (Sagara et al., 1996, Takuma et al., 2004, Watts et al., 2005). There is evidence that an intensive metabolic exchange occurs between astrocytes and neurons which is important in the maintenance of optimal thiol status of neurons and protection of the brain from oxidative stress (Dringen et al., 1999b, Dringen et al., 2000). Indeed, primary murine neurons co-cultured with astrocytes approximately double their intracellular GSH concentration in comparison with neurones grown in monoculture and are thought to be dependent on neighbouring astrocytes for maintenance of their GSH level via provision of cysteine, the rate-limiting substrate for GSH synthesis (Drukarch et al., 1997, Gegg et al., 2003).

The capacity of cells to maintain or even increase glutathione levels during a xenobiotic or oxidative challenge is important in the prevention of cell dysfunction and death (Dringen et al., 2000). It has been suggested that transcriptional up-regulation of glutathione synthesis in astrocytes appears to mediate astrocytic resistance against oxidative stress and enables the astrocytes to protect neurons (Iwata-Ichikawa et al., 1999). The importance of the function of astrocytic GSH metabolism that is evident (at least in cell culture models), suggests that *in vivo* a compromised glutathione system may contribute to a lower defence capacity of the brain against ROS.

Glutathione is synthesised in two stages. Firstly γ-glutamylcysteine is synthesised by the enzyme glutamate cysteine ligase (GCL) from glutamate and cysteine; next, glycine is added by glutathione synthetase (GS). Glutathione is present within cells in both reduced (GSH) and oxidised (GSSG) forms. GSSG can be reduced to GSH by GSSG reductase (GSR). Genes encoding components of the GSH system are activated following binding of the transcription factor nuclear factor-erythroid 2-related factor 2 (Nrf2) to a *cis*-acting DNA promoter sequence called the antioxidant response element (ARE) (Kensler et al., 2007). Expression of the key components of GSH biosynthesis and regulation have been observed in both ESC (Gupta et al., 2012) and iPSC (Chen et al., 2014) derived astrocytes. Upregulation of ESC- derived astrocytic glutathione biosynthesis, secretion, and extracellular breakdown can be used by neurons to support their own glutathione levels thus allowing non-cell-autonomous neuroprotection, a process which has been observed to be maintained through glutathione-dependent and independent mechanisms following treatment with hydrogen peroxide (Gupta et al., 2012). However, in neuron and astrocyte cultures derived from Down's syndrome patients these processes are compromised (Chen et al., 2014). However, the application of a small molecular Nrf2 activator enhanced the neuroprotective effect of human ESC derived astrocytes in these cultures (Gupta et al., 2012). Our laboratory has previously demonstrated that EC derived astrocytes can also modify neuronal toxic responses through GSH and

maintenance of cellular energy levels following treatment with a range of xenobiotics (Woehrling et al., 2010, Woehrling et al., 2007).

Astrocytic metabolism

The abundance of astrocytes, their close proximity to neurons and their position at the interface of blood vessels (Kacem et al., 1998), and synapses, facilitate neuronal metabolic support via intercellular exchange of proteins, lipids and other macromolecules (Naus and Bani-Yaghoub, 1998, Gordon et al., 2007, Iadecola and Nedergaard, 2007). Additionally, astrocytes support neurons via the delivery of nutrients, removal of metabolic waste products and the redistribution of metabolites over long distances (via gap junctions) throughout the astrocytic syncytium (Giaume et al., 2010). Elevated neuronal activity requires an increase in nutrient availability and corresponding shifts in cerebral blood flow (Koehler et al., 2009). Astrocyte end-feet contact the endothelial cells of brain microvessels, thus increasing nutrient delivery to neurons as required (Lopachin and Aschner, 1993).

If cellular ATP production falls due to the inhibition of oxidative metabolism by mitochondrial toxins, it has been postulated that astrocytes, rather than neurons, respond with an increase in glycolytic activity, glucose consumption and lactate production to supplement ATP levels (Almeida et al., 2001). Thus, it is considered that generally astrocytes will demonstrate less vulnerability to mitochondrial toxins than neurones (Almeida et al., 2001, Pellerin and Magistretti, 2003) and that if energy levels of astrocytes can be preserved then their cellular defence mechanisms may reverse or even prevent injury to other brain cells caused by free radical release from ATP depleted cells (Sharma et al., 2003).

The astrocyte-neuron lactate shuttle hypothesis (ANLS)(Pellerin and Magistretti, 1994) provides a potential model to understand how neural activity relates to changes in metabolism and neuronal plasticity (Pellerin and Magistretti, 2012). Glutamate released from neurons into the synaptic cleft is taken up by the glutamate transporters GLAST and GLT-1 and is recycled within astrocytes to produce

glutamine for neuronal use. However, co-transport of Na^+ by glutamate transporters raises intracellular sodium concentrations, activating Na^+/K^+ ATPase which leads to a decrease in cellular ATP. As energy demands increase, glycolysis is enhanced and lactate is released into the extracellular space via the MCT1/4 transporters. Neuronal MCT2 expression allows uptake of lactate which is rapidly converted by LDH1 to pyruvate for ATP generation by the TCA cycle (Pellerin and Magistretti, 2012). During prolonged activity astrocytes may also rely upon reserves of glycogen. Brain glycogen content resides solely in astrocytes and is likely to perform a dynamic role during normal brain function (Obel et al., 2012). Indeed, both potassium and glutamate can promote significant glycogen breakdown, ensuring rapid lactate production during brain activation (Dienel et al., 2002, Swanson, 1992). Glycogenolysis has been shown to be essential in rat hippocampal learning (Suzuki et al., 2011) and chick bead discrimination (Gibbs et al., 2006) and so is intimately linked to memory formation. The pathway involved in the production of lactate by astrocytes in the brain is unclear. Dienel and McKenna (2014) have suggested other metabolic pathways including glutamate oxidation and glycolysis alongside lactate release could also contribute to the energy demands of excitatory neurotransmission.

Our laboratory has recently demonstrated that EC derived neurons and astrocytes display a functional ANLS and that EC derived astrocytes can metabolise glycogen (Tarczyluk et al., 2013). Following neuronal stimulation, astrocytes break down their glycogen and produce more lactate that is released into the surrounding culture media. This process can be blocked using DL-threo-beta-benzyloxyaspartate or ouabain suggesting that astrocytic uptake of glutamate and subsequent activation of the Na^+/K^+ ATPase triggers glycogenolysis and glycolysis in these cells. As metabolic processes are perturbed in neurodegenerative conditions such as Alzheimer's and Parkinson's disease then it is important that the cultures used to model these diseases are able to replicate normal physiology.

Astrocytic involvement in neurotoxicity and disease

Whilst it is well established that the trophic and protective support offered by astrocytes to neurons may provide increased tolerance of neurons to some specific neurotoxins (Yu and Zuo, 1997, Tieu et al., 2001), the multifaceted nature of the astrocytic-neuronal relationship provides numerous potential sites of disruption for neurotoxic chemicals (LoPachin and Aschner, 1993, Tieu et al., 2001). Thus, regarding xenobiotic neurotoxic mechanisms, nerve damage induced by chemicals may not only involve direct damage to the nerve cell (Heijink et al., 2000) but also dissociation or negation of astrocytic-neuronal interactions (LoPachin and Aschner, 1993, Cookson et al., 1995), or damage to the astrocytes themselves (O'Callaghan, 1991, Karpiak and Eyer, 1999). Additionally, there is evidence that astrocytes may be necessary for the expression of neuronal toxic effects, particularly via the release of cytokines from astrocytes (Bruccoleri et al., 1998, Viviani et al., 2000). Cytokines play an important role in regulating the activity of cells in the CNS and serve as an additional means of communication between neurons and astrocytes (Brown, 1999). Cytokines are also important mediators of the host defence system and inflammatory response (Wu and Schwartz, 1998). Astrocytes in the CNS can both secrete and respond to cytokines, such as tumor necrosis factor-alpha (TNF-α). TNF-α release may occur in response to a variety of biological stimuli, including activation (Wu and Schwartz, 1998, Viviani et al., 1998) and may be necessary for the expression of toxicity towards neurons by some substances, for example via induction of the apoptotic cascade (Viviani et al., 1998).

A major function of Glial cells, is to respond dynamically to many CNS pathologies, such as stroke, neurodegenerative disease and exposure to some neurotoxins; indeed damage to all glial cell types, including astrocytes, appears to illicit this glial reactivity (O'Callaghan, 1991, O'Callaghan et al., 1995). This process, also known as astrogliosis or glial activation, is not completely understood but is a hallmark of hypertrophy (O'Callaghan, 1991). Hypertrophy is associated with increased positive staining for GFAP due to an increased number of astrocytic processes, rather than proliferation. Indeed, astrocyte mitosis usually only occurs when a nervous system injury creates a physical space that can be filled by dividing astrocytes (Wu and

Schwartz, 1998). In addition to an increase in GFAP levels, astrocytic activation may be accompanied by increased glucose uptake, and enhanced metabolic status, protein and RNA synthesis (Cookson et al., 1995, Wu and Schwartz, 1998, Pekny and Nilsson, 2005).

In the generation of stem cell derived astrocytes from stem cells it is important to consider the degree of reactivity displayed by the cells produced. In disease modelling reproduction of the reactive state may be essential to observe astrocyte induced toxicity. However, in order to study normal functions in astrocyte cultures, the production of cells which display a mature quiescent astrocytic state is important. Roybon *et al.* (2013) have recently developed protocols that allow the production of both mature quiescent or activated astrocytes that can be used to distinguish between these different functional states. Such methods provide a significant step forward in the ability to study human function in both healthy tissue and in disease.

In addition to their responses to neurotoxins, astrocytes have been implicated in neuroprotection and pathogenesis in numerous neurological conditions (Maragakis and Rothstein, 2006, Sidoryk-Wegrzynowicz et al., 2011) including epilepsy (Benarroch, 2009), ischemia (Anderson et al., 2003), Alzheimer's (Nagele et al., 2004) Parkinson's (Zhang et al., 2005) and Huntington's diseases (Singhrao et al., 1998), as well as Rett syndrome (Ballas et al., 2009), and Amyotrophic Lateral sclerosis (ALS) (Bristol and Rothstein, 1996).

Such findings suggest that modulation of astrocytic function may provide the basis of future novel therapeutic strategies and the exploration of such modalities necessitates the generation of practical and relevant functional human astrocytic models. The development of such new human model systems to study neuron-glia interactions will also advance our understanding of the roles of astrocyte in neurological pathologies. Whilst ESC and EC cells have provided effective model systems for recapitulating normal astrocytic function *in vitro,* their utility in studying disease states may be limited. However, since the generation of induced pluripotent stem cells from somatic cells by ectopic expression of the transcription factors

Oct4, *Sox2*, *Klf4*, and *c-Myc* (Takahashi et al., 2007), the possibility of generating patient specific disease models for neurodegenerative diseases has been realised. Furthermore the technical expertise to directly reprogramme somatic cells to produce induced neural precursor cells (Kim et al., 2011), neuronal cells (Vierbuchen et al., 2010) and induced astrocytes (Caiazzo et al., 2015) has further expanded the toolkit available to researchers. Interestingly, one of the deficiencies in early approaches to modelling specific neurodegenerative diseases in vitro has been a lack of neuronal and astrocytic cultures with the specific anatomical and functional characteristics of the particular brain tissues affected. However, this has been remedied through the recent development of neural differentiation protocols that allow the formation of region- specific neuronal cultures including dopaminergic, spinal cord, interneurons and cortical neurons to be produced (Chambers et al., 2009, Fasano et al., 2010, Li et al., 2005, Liu et al., 2013, Shi et al., 2012).

Indeed, the use of iPSC platforms has rapidly expanded and they have been successfully applied to the generation of a wide range of disease specific neuronal platforms including ALS (Dimos et al., 2008), Huntington's (Zhang et al., 2010), familial dysautonomia (Lee et al., 2009), spinal muscular atrophy (Ebert et al., 2009), Rett syndrome (Marchetto et al., 2010), schizophrenia (Brennand et al., 2011), Alzheimer's (Kondo et al., 2013, Israel et al., 2012) and Parkinsons's disease (Devine et al., 2011). These models not only allow researchers to study disease pathology directly, but also allow rapid screening of novel potential therapeutic compounds in the same directly relevant model to man.

Paradoxically, many of these studies have focused on the sole production of neurons in these cultures and have overlooked the role of astrocytes. However, a number of recent studies have demonstrated the production highly purified populations of astrocytes (Krencik and Zhang, 2011, Krencik et al., 2011, Serio et al., 2013, Shaltouki et al., 2013, Juopperi et al., 2012). Such *in vitro* models allow researchers to study the pathology of patient- derived astrocytes as well as to demonstrate the non-cell autonomous effects on healthy neurons in a number of diseases. For

example, using astrocytes derived from Rett syndrome patients' iPSCs, several key abnormalities have been revealed, in comparison with healthy cells, with regard to astrocyte differentiation, dysregulated GFAP expression, as well as abnormal non-cell autonomous effects on the morphology and function of heathy neurons (Williams et al., 2014, Andoh-Noda et al., 2015). In addition, other groups have also recapitulated increased vacuolation phenotypes in Huntington's disease patient derived astrocytic cells (Juopperi et al., 2012). Using ALS patient derived cells, TAR DNA-binding Protein 43 mutants demonstrated increased levels of TDP-43 mislocalisation and decreased astrocyte survival (Serio et al., 2013). Furthermore, Meyer *et al*. (2014) used induced neural precursor cells from patients carrying the hexanucleotide expansion in C9ORF72 that has also been associated with ALS and FTD. Following differentiation into astrocytes these cells displayed non-cell autonomous toxicity towards motor neurons in a manner previously reported for cells derived from autopsies. In cells derived from Down's syndrome patients, astrocytes display higher levels of ROS as well as non-cell autonomous effects on neurons, including reduced neurogenesis, ion channel maturation and synapse formation. (Chen et al., 2014). This study also demonstrated the partial correction of pathological phenotypes using the drug minocycline. Such studies suggest a potential role of astrocytes in these disease processes and provide potential platforms for high-throughput drug screening as well as mechanistic studies that may highlight future therapeutic approaches.

Conclusion

In this review we have focussed upon the role of astrocytes in normal functioning and disease within the CNS. Whilst there is a rapid expansion in the use of iPSC technology to study human neurodegenerative disease, the inclusion/role of astrocytes in this context has often been overlooked. A growing body of work has demonstrated the feasibility of generating functional, disease-relevant astrocytes from iPSCs. The rapid development of patient- derived iPSC lines is an exciting step in studying numerous developmental and neurodegenerative disorders. However, in

order to fully realise the future role of these models, further characterisation of these cultures and inclusion of functional astrocytes is essential. Furthermore a deeper understanding of astrocytic diversity and their functional roles within the CNS is necessary in order to develop realistic neuronal circuits that will enable the elucidation of their functional role in health and disease. The identification of factors involved in the patterning of specific astrocytic subtypes *in vitro* is also required to provide cultures that are representative of astrocytic heterogeneity (Khakh and Sofroniew, 2015). In addition, improvements in culture conditions are also important to produce relevant cell types as well as neural circuits, which may only be realised using 3D cultures that recapitulate the in vivo environment (Lancaster et al., 2013).

Existing *in vitro* and *in vivo* experimental models have given us a tantalizing glimpse of the complexity of the different forms and functions of the human astrocyte. Further development of these technologies will enable us gain a greater understanding of normal astrocytic functions as well as their role in disease processes that will ultimately expedite the process of drug discovery.

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Competing interests

The authors disclose no conflict of interest.

Author contributions

The authors have contributed equally to the preparation of this manuscript.

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Figure Legend

Abstract figure

Astrocytic cells have been derived from a variety of stem cell sources. This review discusses the roles of astrocytes in the brain and highlights the extent to which human stem cell derived astrocytes have demonstrated functional activities that are equivalent to that observed *in vivo*.

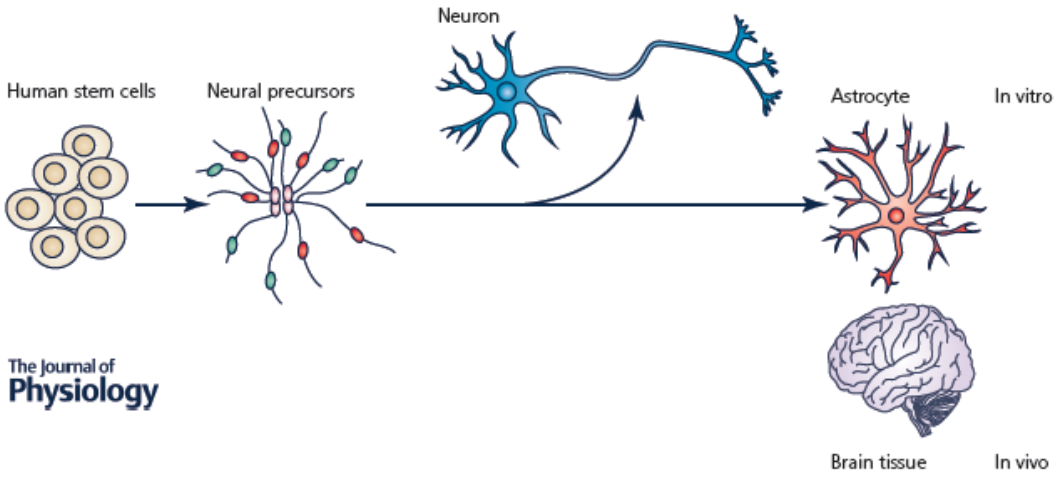

Fig 1. Properties of stem cell derived astrocytes. Astrocytic cells derived from stem cells have been shown to recapitulate features previously observed *in vivo*. 1) Glutamatergic transmission, 2) Metabolism, 3) Oxidative stress, 4) Disease states, 5) Development, 6) Gliotransmission.