Properties of human astrocytes and NG2 glia

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Abstract
Since animal models are inevitable for medical research, information on species differences in glial cell properties is critical for successful translational research. Here, we review current knowledge about morphological and functional properties of human astrocytes and NG2 glial cells and compare these data with those obtained for the comparable cells in rodents. Morphological analyses of astrocytes in the neocortex of rodents versus humans have demonstrated clear differences. In contrast, the functional properties of astrocytes or NG2 glial cells in these species are surprisingly similar. However, these findings should be interpreted with caution, as so far functional analyses of human cells are only available from neocortex and hippocampus, and it is known from rodent studies that the properties of astrocytes in different brain regions may vary considerably. Moreover, technical challenges render astrocyte electrophysiological measurements in situ unreliable, and human cell properties may be affected by medications. Nevertheless, based on the limited data currently available, there is substantial similarity between human and rodent astrocytes with regard to those functional properties studied to date. The unique morphological characteristics of astrocytes in human neocortex call for further physiological analysis. The basic properties for NG2 glia are even less completely evaluated with regard to the question of species differences but no glaring differences have been reported so far. In conclusion, it remains justifiable to employ mouse or rat models to investigate the etiology of human CNS diseases that might involve astrocytes or NG2 glia.

KEYWORDS
astrocyte, electrophysiology, human, morphology, mouse, NG2 glia, rodent, species differences

1 | INTRODUCTION

Glial cells were first described by Rudolf Virchow in middle of the 18th century as "Nervenkitt" (nerve putty/cement/glue), because he assigned them merely structural functions (Virchow, 1856; Virchow, 1859). The term "astrocyte" was proposed almost 50 years later by Michael von Lenhossék and refers to the star-shaped morphology of the cells (Lenhossék, 1893). Today, we know that astrocytes comprise a morphologically and functionally highly heterogeneous class of cells, which makes their identification difficult (Griemsmann et al., 2015; Matyash & Kettenmann, 2010; Seifert & Steinhäuser, 2018). Common characteristics include the absence of electrical excitability, a high resting K⁺ conductance and therefore a membrane potential close to the K⁺ equilibrium potential, extensive connections to each other via gap junctions, expression of functional glutamate and GABA transporters, formation of numerous fine processes enwrapping synapses and blood vessels,
and expression of intermediate filament proteins such as GFAP and vimentin (Verkhratsky & Parpura, 2015). Research over the past decades revealed that astrocytes are way more than “nerve glue” as they fulfill a plethora of vital physiological functions and, consequently, are also key players in neurological diseases (Parpura et al., 2012; Seifert, Schilling, & Steinhäuser, 2006; Verkhratsky & Parpura, 2015). Their functions include supply of nutrients to neurons, control of extracellular ion homeostasis, clearance of neurotransmitters, regulation of the blood–brain barrier (BBB) permeability, promotion of synapse formation, and contribution to the immune response by release of proinflammatory cytokines or neurotrophic factors (Verkhratsky & Parpura, 2015). Importantly, astrocytes also directly modulate synaptic transmission by release, uptake, degradation, and recycling of transmitters (Araque et al., 2014; Verkhratsky & Nedergaard, 2018). To accomplish this, the fine processes of individual astrocytes may ensheathe >100,000 synapses (Bushong, Martone, Jones, & Ellison, 2002). The dynamic regulation of the presynaptic and postsynaptic by closely associated glial processes led to the concept of the “tripartite synapse” (Araque, Parpura, Sanzgiri, & Haydon, 1999). According to this concept, astrocytes not only detect and react to neuronal activity but also respond to and regulate neuronal activity and plasticity through the release of neuroactive substances (so-called gliotransmitters), such as glutamate, ATP, and D-serine (Bezzi et al., 1999). The in vivo relevance of this process, termed gliotransmission, is, however, still matter of debate (Fiocco & McCarthy, 2018; Savtchouk & Volterra, 2018). A striking feature of astrocytes is that they are electrically and metabolically coupled to each other through connexin channels, leading to the formation of large syncytium-like functional networks. Due to this network organization, they are in a position to effectively control and synchronize large neuronal assemblies (Giaume, Koulaïkoff, Roux, Holcmans, & Rouach, 2010).

Besides astrocytes and oligodendrocytes, NG2 glial cells emerged during the last decades as a third type of macroglial cells in the CNS (Nishiyama, Komitova, Suzuki, & Zhu, 2009; Peters, 2004). These cells, also referred to as oligodendrocyte precursor cells, complex cells, synthetocytes, polydendrocytes, or GluR cells (Steinhäuser, Jabs, & Kettenmann, 1994; for review see Bergles, Jabs, & Steinhäuser, 2010; Matyash & Kettenmann, 2010) exhibit both neuronal and glial properties. For instance, they express functional voltage-gated K+, Na+, and Ca2+ channels but are unable to generate action potentials (Haberlandt et al., 2011; Nishiyama et al., 2009). Glial cells expressing the proteoglycan NG2 are abundantly distributed in white and gray matter areas of the developing and adult brain, where they make up 5–10% of all glia and are heterogeneous with regard to their antigen profile (Degen et al., 2012; Mosshrefi-Ravasdjani et al., 2017; Trotter, Karram, & Nishiyama, 2010). They sustain the capacity to proliferate and differentiate throughout life and constitute therefore the largest proliferative cell population outside the adult neurogenic niches in the CNS (Dawson, Polito, Levine, & Reynolds, 2003; Mosshrefi-Ravasdjani et al., 2017). Fate mapping studies revealed that in the postnatal white matter, NG2 glial cells mainly differentiate into myelinating oligodendrocytes (Dimou, Simon, Kirchhoff, Takebayashi, & Götz, 2008; Kang, Fukaya, Yang, Rothstein, & Bergles, 2010; Zhu et al., 2011; Zhu, Bergles, & Nishiyama, 2008), a process that is enhanced following demyelination, CNS injury, and in chronic neurodegenerative disease (Kang et al., 2010; Levine & Reynolds, 1999; Magnus et al., 2008; McTigue, Wei, & Stokes, 2001). In contrast, most of the grey matter NG2 glia do not differentiate into oligodendrocyte but maintain their phenotype throughout postnatal life (Dimou et al., 2008; Rivers et al., 2008; Zhu et al., 2008). In several brain regions, the density of NG2 glia decreases with increasing age (Mosshrefi-Ravasdjani et al., 2017) and their loss through death, differentiation, or ablation induces rapid proliferation and migration of adjacent cells to reestablish their density (Hughes, Kang, Fukaya, & Bergles, 2013). Intriguingly, rodent NG2 glia receive direct synaptic inputs from glutamatergic and GABAergic neurons, a feature that is unique among glial cells (Bergles, Roberts, Somogyi, & Jahr, 2000; Jabs et al., 2005; Lin & Bergles, 2004). The function of these neuron–glia synapses, whose existence has been confirmed by electron microscopy (Bergles et al., 2000; Haberlandt et al., 2011; Jabs et al., 2005; Lin & Bergles, 2004) and which, fascinatingly, were shown to undergo an NMDA-independent form of long-term potentiation (Ge et al., 2006), is still enigmatic. Thus, NG2 glia constitute a distinct class of glial cells, but their function is poorly understood.

2 MORPHOLOGICAL PROPERTIES OF HUMAN Astrocytes AND NG2 GLIA

2.1 Astrocytes

Morphological studies revealed that astrocytes in the human cortex are larger, more complex and more diverse than those in rodents (Oberheim et al., 2009; Oberheim, Wang, Goldman, & Nedergaard, 2006; Vasily, Dossi, & Rouach, 2017). In addition, fibrous astrocytes in white matter and protoplasmic astrocytes in grey matter, two morphologically distinct subtypes of GFAP-positive cells, interlaminar and varicose projection astrocytes, are exclusively found in the cortex of higher primates (Colombo & Reisin, 2004; Colombo, Aíñez, Puissant, & Lipina, 1995; Oberheim et al., 2009; Susunov et al., 2014). Human interlaminar astrocytes are solely found in the cortical layer I. They have small spheroid cell bodies with numerous short processes and one or two very long, tortuous processes that terminate in the neuropil or vasculature of cortical layer II to IV (Oberheim et al., 2009). The function of these cells is yet undefined, although it has been speculated that they are involved in information transfer between cortical layers (Oberheim et al., 2009). Varicose projection astrocytes, the second primate-specific subtype of astrocytes, are rather sparsely found in cortical layers V–VI. They typically exhibit several relatively short spiny processes and 1- to 5-mm-long unbranched processes with regularly spaced varicosities that extend within the deep cortical layers. These cells were hypothesized to mediate long-distance communication across cortical layers or even between gray and white matter (Oberheim et al., 2009). Human cortical GFAP-positive protoplasmic astrocytes were reported to have 10 times more (37.5 vs. 3.8) and 2.6-fold longer (97.9 vs. 37.2 μm) primary processes than their rodent
counterparts. Consequently, their domain diameter is about 2.6 times larger (142.6 vs. 56 μm), which corresponds to a 16.5-fold greater occupied volume. In contrast, the cell body of cortical astrocytes seems to be of comparable size in rodents and man (Oberheim et al., 2009; Oberheim Bush & Nedergaard, 2017), although we could not find where this data was originally published. Due to the larger diameter and the associated enhanced complexity of secondary process arborisation, human astrocytes were estimated to contact up to 2 million synapses, compared with up to 140,000 in rodents (Bushong et al., 2002; Oberheim et al., 2009). Like in rodents, cortical human astrocytes are organized in domains with little overlap, although the average area of overlap is greater in humans than in rodents (204.7 vs. 118 μm²) (Oberheim et al., 2008; Oberheim et al., 2009). Human fibrous astrocytes in the white matter were also reported to be larger in the human versus mouse brain (183.2 vs. 85.6 μm) (Oberheim et al., 2009).

No morphometric analyses of astrocytes have yet been performed in human hippocampus. Thus, we have further evaluated GFAP/S100β double staining performed previously in nonsclerotic hippocampal specimens from temporal lobe epilepsy (TLE) patients (Bedner et al., 2015) and compared the data with those obtained from mouse hippocampus (Degen et al., 2012). The results revealed that soma volume and derived diameter of human hippocampal astrocytes are larger than those of mice (Figure 1).

2.2 | NG2 glia

The morphological properties of human NG2 glia are less well characterized. In rodents, gray matter NG2 glia display a stellate appearance with a central round soma from which they extend several radial processes that are, however, much less branched than astrocytic processes (Jabs et al., 2005; Nishiyama, Yang, & Butt, 2005). In white matter the somata of NG2 glial cells are more elongated and the processes orientated along axonal projections (Chittajallu, Aguirre, & Gallo, 2004). In addition to the proteoglycan NG2, in rodents they coexpress the platelet-derived growth factor-α (PDGFα) receptor (Nishiyama, Lin, Giese, Heldin, & Stålcup, 1996).

Human NG2-positive glial cells were first described in tissue sections from adult neocortex and white matter by Chang, Nishiyama, Peterson, Prineas, and Trapp (2000) and Dawson, Levine, and Reynolds (2000). The former study reported that morphology and distribution of NG2 glia are similar in the human and rodent brain. The latter study, however, indicated that human NG2 glia have fewer processes than their rodent counterparts, an observation that apparently has not been quantified or confirmed later on (Dawson et al., 2000). Like in rodents, human NG2 glia consistently coexpress NG2 and PDGFα receptors but not GFAP (Bedner et al., 2015; Wilson, Scolding, & Raine, 2006). In contrast, the Ca²⁺ binding protein S100β was expressed in almost all NG2 glial cells of human hippocampus (Bedner et al., 2015), but only in a subset of mouse hippocampal NG2 glia (Jabs et al., 2005; Karram et al., 2008; Moshefri-Ravasjani et al., 2017). Interestingly, in the human nonsclerotic hippocampus the density of NG2 glial cells was higher than in the hippocampus of mice (~11 × 10³ vs. ~1.5 × 10³ cells/mm³). Moreover, the density of NG2 glia in the human tissue was similar to that of astrocytes, while mice display an NG2 glia/astrocyte ratio of about 1:4 in the same brain region (Bedner et al., 2015; Degen et al., 2012). It is, however,
possible that NG2 glial cell numbers in human tissue were influenced by seizures the patients experienced and, therefore, not representa-
tive for the situation in the healthy human brain. Indeed, seizure-
induced proliferation of NG2 glial cells has been demonstrated in
an experimental seizure model (Wennström, Hellsten, Ekdahl, &
Tingström, 2003). As no information on the cell size of human NG2
glia is yet available, we further analyzed previously performed
PDGFRα staining in the nonsclerotic human hippocampus (Bedner
et al., 2015) and compared the data with fluorescently labeled murine
NG2 glia of the same region (CA1 stratum radiatum; Degen et al.,
2012). Similar to astrocytes, the somata of human NG2 glial cells were
larger as those of their rodent counterparts (Figure 1). Interestingly,
the analysis further revealed that only in mice, the soma volume and
diameter of NG2 glia exceeds those of astrocytes (Figure 1).

3 | FUNCTIONAL PROPERTIES OF HUMAN
ASTROCYTES AND NG2 GLIA

3.1 | Methods to study human glial cells

Data on functional properties of human glial cells are sparse due to
the limited availability of healthy human tissues and technical difficul-
ties with recordings from human slices. Most analyses have been per-
formed on acute brain slices surgically resected from patients with
medically intractable TLE. Here, the experiments were either per-
formed in acute slices from cortical specimens resected to gain access
to the epileptogenic area (Navarrete et al., 2013; Oberheim et al.,
2009), or in nonsclerotic hippocampal slices from patients with
“lesion-associated” TLE that lacked significant histopathological hip-
 pocampal alterations (Bedner et al., 2015; Heinemann et al., 2000;
Hinterkeuser et al., 2000; Jauch, Windmüller, Lehmann, Heinemann,
& Gabriel, 2002; Kivi et al., 2000; Navarrete et al., 2013; Seifert et al.,
2002; Seifert, Hüttmann, Schramm, & Steinhäuser, 2004). In the latter,
seizures are generated by focal lesions in the temporal lobe, such as
tumors (e.g., gangliogioma or dyssembryoplastic neuroepithelial
tumors), malformations of cortical development (mainly focal cortical
dysplasia) or vascular malformations (Blümcke et al., 2017). Although
these specimens display anatomically preserved structures and lack
significant neuropathological changes, it must be considered that sei-
zure activity and/or medication may have affected cellular properties.
Besides tissue from epilepsy surgery, biopsy samples from tumor
patients without epileptic seizures were used for functional analysis
of cortical astrocytes (Bordey & Sontheimer, 1998; Oberheim et al.,
2009; Picker, Pieper, & Goldring, 1981). However, such specimens are
rare, and though cells in tissue outside the tumor margins are consid-
ered functionally unaffected, tumor- or medication-associated alter-
ations cannot be completely ruled out. Moreover, biopsies are
obtained from different cortical subareas, which may limit comparabil-
ity of the results.

Numerous studies have investigated properties of human glial
cells in vitro, that is, in primary or induced pluripotent stem (iPS)
cell-derived cell cultures. However, it is well established that the proper-
ties of primary glial cultures significantly differ from cells in acute
preparations or in vivo. The iPS cell technology provides a potentially
unlimited source of various human cell types and opens new avenues
for biomedical research and drug discovery. However, to what extent
iPS cell-derived cells resemble their counterparts in vivo, is still
unclear. The absence of the cells’ native environment, as well as
potential epigenetic changes and different developmental timelines,
may influence the features of these cells. Thus, we have focused our
review on functional data gathered from in situ studies.

3.2 | Electrophysiological properties of human
astrocytes

In rodents, adult astrocytes are characterized by a high resting K+ con-
ductance, giving rise to a very low input resistance (Ri) (<5 MΩ), a rest-
ing potential close to the equilibrium potential of K+ (EK) and almost
linear (“passive”) current to voltage relationships (Jabs, Seifert, &
Steinhäuser, 2008; Verkhratsky & Parpura, 2015). First whole cell
patch-clamp recordings from glial cells in acute human cortical and
hippocampal specimens were performed by Bordey and Sontheimer
(1998) and Hinterkeuser and colleagues (Hinterkeuser et al., 2000).
However, the two studies cannot be directly compared because
(a) different K+ concentrations ([K+]o) were used in the pipette and bath
solutions, which affects resting potentials and (b) the age of the
patients analyzed varied greatly (4 months to 14 years vs. mean age
of 34 years), which may explain the higher Ri of cells in the former
study (Ri = 288 MΩ vs. 140 MΩ) for developmental changes in Ri see
Kressin, Kuprijanova, Jabs, Seifert, & Steinhäuser, 1995). Referring to
these two studies, it has been speculated that an apparently higher
Ri in human versus rodent astrocytes represents an evolutionary adapta-
tion to the larger size of astrocytes, as it results in an increased length
constant (Oberheim Bush & Nedergaard, 2017; Vasile et al., 2017).
However, the whole cell current patterns shown in the papers by
Bordey and Sontheimer (1998) and Hinterkeuser et al. (2000) indicate
that the authors had analyzed NG2 glial cells and not astrocytes. In
fact, at the end of the 1990s and beginning of the 2000s, glial cells
with time- and voltage-dependent transmembrane currents were still
erroneously regarded as immature astrocytes (discussed in Bergles
et al., 2010). This conclusion is strongly supported by a later study
that systematically compared properties of hundreds of bona fide
astrocytes and NG2 glial cells in “control-like” (i.e., nonsclerotic) hip-
 pocampi from patients with pharmacoresistant TLE. In this study,
human astrocytes always showed predominating passive currents lack-
ing time- or voltage-dependence, similar to their rodent counterpart
(Bedner et al., 2015). Importantly, by further evaluating recordings
from our latter study, we determined an average Ri of human astro-
cytes of 4.6 ± 3 MΩ (n = 21 unpublished), which is in the range of Ri
of rodent astrocytes (juvenile: 8.1 ± 6.5 MΩ, n = 38, Jabs et al., 2005;
adult: 3.3 ± 0.9 MΩ, n = 63; unpublished). The resting potential of
human astrocytes was −67.9 ± 4.5 mV (liquid junction potential not
compensated for, unpublished observation), which is comparable to
that in mouse astrocytes of the same region (juvenile: −82 ± 4 mV,
n = 38, Jabs et al., 2005; adult: −90.3 ± 0.5 mV, Wallraff et al., 2006,
n = 19) considering that with the lower [K+]o (3 mM in Jabs et al.,
NG2 glial cells express voltage-gated A-type (KA) and delayed rectifier and Schröder et al. (2000) furthermore demonstrated that human neurons. Current injections into human NG2 glia never produced are about an order of magnitude lower than those typically seen in rat hippocampus (16 pA/pF; Xie et al., 2007). These current densities Kressin, Kuprijanova, Weber, & Seifert, 1994; De Biase et al., 2010) or matching data reported from mouse (10

Together, these findings indicate that the membrane properties of astrocytes are largely conserved between rodents and humans.

3.3 | Electrophysiological properties of human NG2 glia

The functional properties of NG2 glial cells are unique among glia, as they express various types of ligand- and voltage-gated ion channels typically attributed to neurons (Bergles et al., 2010; Larson, Zhang, & Bergles, 2016). In rodents, the resting potential of NG2 glia is very negative (i.e., close to $E_K$) due to a high $K^+$ conductance at negative voltages (Larson et al., 2016). However, the density of these channels seems to be lower than in astrocytes, as NG2 glia have a substantially higher $R_i$. The resting $K^+$ conductance (mainly mediated by Kir4.1 channels; Tang, Taniguchi, & Kofuji, 2009) increases during postnatal development (Bordey & Sontheimer, 1997; Kressin et al., 1995; Maldonado, Vélez-Fort, Levavasseur, & Angulo, 2013), producing more passive whole cell current patterns and a lower membrane resistance in adult rodents (~30–50 MΩ in adults vs. ~100–500 MΩ in juveniles; Kressin et al., 1995; Lin & Bergles, 2004; Kukley et al., 2008; Haberlandt et al., 2011; Braganza et al., 2012; Maldonado et al., 2013; Larson et al., 2016; Passlick, Trotter, Seifert, Steinhäuser, & Jabs, 2016). Although NG2 glial cells receive synaptic input and express almost the same set of voltage-gated ion channels as neurons, they are not able to fire action potentials, due to the relatively low density of voltage-gated Na$^+$ (Na$^+$) channels (De Biase, Nishiyama, & Bergles, 2010; Larson et al., 2016).

Hinterkeuser et al. (2000) have functionally characterized human NG2 glia in the histopathologically intact human hippocampus resected from patients with lesion-associated TLE (although at that time, these cells were still thought to be immature astrocytes; see Section 3.2). The human cells exhibited mean $R_i$ (140 MΩ) and $C_m$ (26 pF) values similar to those determined in rodent NG2 glia (Larson et al., 2016). About 75% of the human cells displayed TTX-sensitive Na$^+$ currents, with an average density of 17 pA/pF, also closely matching data reported from mouse (10–30 pA/pF Steinhäuser, Kressin, Kuprijanova, Weber, & Seifert, 1994; De Biase et al., 2010) or rat hippocampus (16 pA/pF; Xie et al., 2007). These current densities are about an order of magnitude lower than those typically seen in neurons. Current injections into human NG2 glia never produced action potentials, similar to rodent NG2 glia. Hinterkeuser et al. (2000) and Schröder et al. (2000) furthermore demonstrated that human NG2 glial cells express voltage-gated A-type ($K_a$) and delayed rectifier ($K_{Ca}$) $K^+$ channels as well as inward-rectifier Kir4.1 $K^+$ channels, similar to what has been found in rodents (Larson et al., 2016). Although Hinterkeuser et al. (2000) performed the measurements in hippocampal NG2 glia from adult patients (average age 34 years), the density of inwardly rectifying $K^+$ currents was apparently lower than in cells from adult mouse hippocampus (Braganza et al., 2012). However, this difference might have been due to different protocols used for Kir current isolation (peak current subtraction has been performed in the former study). A large number of NG2 glia in the nonsclerotic human hippocampus was recently characterized by Bedner et al. (2015). The authors demonstrated that human hippocampal NG2 glia (a) lack gap junction coupling, (b) express ionotropic glutamate receptors, and (c) lack glutamate transporter (Figure 2), with these properties exactly matching characteristics of this cell type in mouse hippocampus (Matthias et al., 2003; Wallraff, Odermatt, Willecke, & Steinhäuser, 2004) (see in the following). Further evaluation of the recordings obtained by Bedner et al. (2015) revealed similar values for $R_i$ (124.2 ± 80 MΩ) and $C_m$ (24.3 ± 10.2 pF; n = 111 cells from 25 patients; unpublished observation) as in the earlier study by Hinterkeuser et al. (2000) and confirmed that there are no species-dependent differences in these parameters between man and mouse. Intriguingly, further evaluation of the measurements obtained by Bedner et al. (2015) indicated that human NG2 glial cells also undergo developmental changes in their ion channel expression patterns. Indeed, in hippocampal NG2 glia of patients younger than 14 years of age, $R_i$ was substantially higher and the density of Kir currents lower than in tissue from older patients (age > 14 years: $R_i$ = 83.3 ± 34.1 MΩ; $C_{DKIR}$ = 41.6 ± 19.4 pA/pF; $n$ = 49 cells from 14 patients vs. age < 14 years: $R_i$ = 176.1 ± 92.7 MΩ, $C_{DKIR}$ = 27.4 ± 11.7 pA/pF; $n$ = 62 cells from 11 patients; unpublished observation). Hence, human NG2 glial cells display similar developmental changes in basic membrane properties like their rodent counterparts. Furthermore, the density of Kir currents in adult human NG2 glia (as calculated from the recordings of Bedner et al., 2015) did not differ from hippocampal NG2 glia of adult mice (31.1 ± 3 pA/pF; $n$ = 9; Braganza et al., 2012). Mean peak outward current densities of human hippocampal NG2 glia determined from the data of Bedner et al. (2015) displayed no developmental changes and were similar to those reported by Hinterkeuser et al. (2000).

Together, these data demonstrate that basic membrane properties, that is, ion channel expression profiles, and its developmental regulation, of NG2 glia in rodent and human hippocampus do not differ.

3.4 | Glutamate sensitivity of human astrocytes and NG2 glia

One of the major roles of astrocytes is clearance of excessive glutamate from the synaptic cleft, a pivotal mechanism for normal excitatory neurotransmission and protection against excitotoxicity (Schoosboe, Scafidi, Bak, Waagepetersen, & McKenna, 2014). This is accomplished via specific transporters, called excitatory amino acid transporters (EAATs), which are enriched in perisynaptic astrocyte membranes and utilize the electrochemical gradient of Na$^+$ and K$^+$ as
a driving force for transmembrane movement of glutamate. Astrocytes express EAAT1 and EAAT2, often referred to as glutamate-aspartate transporter 1 (GLAST1) and glutamate transporter 1 (GLT-1) in rodents (Vandenberg & Ryan, 2013). Although several studies have investigated expression of EAAT protein and transcripts in the human brain (e.g., Bjørnsen et al., 2007; Roberts, Roche, & McCullumsmith, 2014), their functionality in situ has been examined only recently (Bedner et al., 2015). In the latter study, rapid application of glutamate to outside-out patches excised from the soma of astrocytes in non-sclerotic human hippocampus evoked transient inward currents at negative membrane potentials, which were completely inhibited by the glutamate transporter blocker DL-TBOA, but were insensitive to the AMPA/kainate receptor antagonist NBQX, indicating that the observed currents were due to glutamate uptake through EAAT transporters (Figure 2; Bedner et al., 2015). NG2 cells in rodent hippocampus express functional AMPA and GABA<sub>A</sub> receptors and receive direct synaptic input from neurons (Bergles et al., 2000; Jabs et al., 2005; Lin & Bergles, 2004). AMPA receptors are tetramers formed by the subunits GluA1–4 which, dependent on their subunit composition, considerably vary in their functional properties. In mouse hippocampal NG2 glia, transcripts of all four subunits were detected, with co-expression of GluA1, GluA2, and GluA4 being most frequent (Seifert, Weber, Schramm, & Steinhäuser, 2003; Seifert, Zhou, & Steinhäuser, 1997). In the juvenile hippocampus, a mixture of AMPA receptors with high and low Ca<sup>2+</sup> permeability coexist in individual cells, while receptors in NG2 glia from older animals are more uniform and display a lower divalent cation permeability (Seifert et al., 2003). AMPA receptor subunits occur in two splice variants, called flip and flop, which determine the gating properties of the receptors. The GluA2 flip variant is upregulated during maturation of hippocampal NG2 glia (Seifert et al., 2003). It has been shown that sustained activation of AMPA receptors in NG2 glial cells of mouse hippocampus inhibits Kir currents (Schröder, Seifert, Hüttmann, Hinterkeuser, & Steinhäuser, 2002). NG2 glia in human hippocampal tissue resected from patients with intractable TLE also express functional AMPA receptors (Bedner et al., 2015; Seifert et al., 2002; Seifert et al., 2004). Indeed, bath application of the receptor agonist kainate in situ produced receptor currents in the human cells that were completely inhibited by the specific AMPA receptor antagonist, GYKI53655. This antagonist also blocked the glutamate-induced responses in acutely isolated cells, indicating that human NG2 glia, like their rodent counterparts (Matthias et al., 2003), selectively express ionotropic glutamate receptors of the AMPA subtype (Seifert et al., 2004). Species-dependent differences were detected in AMPA receptor desensitization kinetics, which was faster in human than in mouse NG2 glia. Variable splicing of the receptor subunits might account for this difference, as the GluA1 flip/flop splice variant ratio was considerably lower in human versus mouse NG2 glia (Seifert et al., 2003; Seifert et al., 2004). In a later study, the glutamate sensitivity of human hippocampal NG2 glial cells was investigated through flash photolysis of caged glutamate in FIGURE 2 Characterization of astrocytes and NG2 glial cells in the nonsclerotic human hippocampus. (a) The whole-cell current pattern of an astrocyte (left; 50-ms voltage steps ranging from −160 to +20 mV; 10 mV increments; V<sub>hold</sub> = −80 mV) was dominated by a passive resting conductance. Rapid application of glutamate to an outside-out patch failed to induce outward currents at positive voltages (middle and right), indicating the absence of ionotropic receptors. The inward currents were due to glutamate uptake. (b) Gap junction coupling between hippocampal astrocytes visualized by diffusion of biocytin from a single cell, filled with the tracer through the patch pipette during 20 min of whole cell recording. Scale bar = 100 μm. (c) Typical whole-cell current pattern of an NG2 glial cell (left). Depolarization and hyperpolarization activated time- and voltage-dependent outward and inward currents. Flash photolysis of caged glutamate activated currents with a linear current/voltage relationship (middle and right) indicating expression of ionotropic receptors and lack of glutamate transporters. (d) Biocytin filling of NG2 glial cells revealed lack of tracer coupling. Scale bar = 25 μm. Modified from Bedner et al. (2015), reproduced with permission.
Astrocytes in the rodent brain are interconnected through gap junctions that enable intercellular exchange of ions, second messengers, nutritional metabolites, nucleotides, amino acids, small peptides, and RNA (Harris, 2007). Astrocyte coupling in mouse hippocampus predominately depends on gap junction channels composed of connexin43 (Cx43) protein and only to a minor extent on Cx30-based channels (Gosejacob et al., 2011). The astroglial coupling network possesses important functions, including spatial buffering of Ca$^{2+}$ waves (Gosejacob et al., 2011). The first characterization of functional gap junctional coupling between human NG2 glia and astrocytes in the human and mouse hippocampus display the same segregated expression of glutamate receptors and transporters (Matthias et al., 2003).

### 3.5 Gap junction coupling between human astrocytes

Astrocytes balance extracellular K$^+$ levels mainly by two mechanisms, K$^+$ uptake and spatial buffering. Net uptake of K$^+$ is mainly mediated by Na$^+$/K$^+$ ATPases and Na$^+$–K$^+$–Cl$^-$ cotransporters and accompanied by cell swelling and local depolarization of astrocytes (D’Ambrosio, Gordon, & Winn, 2002; Kofuji & Newman, 2004; Ransom, Ransom, & Sontheimer, 2000; Steinhäuser, Seifert, & Bedner, 2012). A very effective and energy independent mechanism of glial K$^+$ clearance describes the spatial buffering hypothesis, according to which excessive extracellular K$^+$ is taken up by astrocytes at sides of high neuronal activity, and then redistributed through the astrocytic gap junction-coupled network to be released at distant regions of lower [K$^+$]o. Here, uptake and release of K$^+$ occur passively, via diffusion through Kir4.1 channels (Kofuji & Newman, 2004; Orkand et al., 1966; Walz, 2000).

The ability of human astrocytes to buffer extracellular K$^+$ through Kir channels has already been investigated almost 20 years ago (Heinemann et al., 2000; Jauch et al., 2002; Kivi et al., 2000). In these studies, the authors used ion-selective microelectrodes to assess the effect of Ba$^{2+}$, which blocks glial Kir channels at sub-mM concentrations, on rises in [K$^+$]o, induced either by repetitive alvear stimulation or by iontophoretic application of K$^+$ in hippocampal tissue from patients with intractable TLE. In the nonsclerotic human hippocampus, even μM concentrations of Ba$^{2+}$ substantially augmented [K$^+$]o, rises induced by both methods, suggesting a significant contribution of glial Kir channels to K$^+$ clearance (Heinemann et al., 2000; Jauch et al., 2002; Kivi et al., 2000). Together with the above-mentioned findings that human astrocytes possess a high resting K$^+$ conductance and are abundantly interconnected through gap junctions, these results argue in favor of a crucial involvement of spatial K$^+$ buffering in removal of external K$^+$ in the human hippocampus.

### 3.6 K$^+$ buffering by human astrocytes

During neuronal activity, K$^+$ is released into the extracellular space where its concentration transiently increases. Since extracellular accumulation of K$^+$ would lead to neuronal depolarization and hyperexcitability, tight control of K$^+$ homeostasis has been hypothesized as a major function of astrocytes. K$^+$ uptake and spatial buffering. The uptake of K$^+$ is mainly mediated by Na$^+$/K$^+$ ATPases and Na$^+$–K$^+$–Cl$^-$ cotransporters and accompanied by cell swelling and local depolarization of astrocytes (D’Ambrosio, Gordon, & Winn, 2002; Kofuji & Newman, 2004; Ransom, Ransom, & Sontheimer, 2000; Steinhäuser, Seifert, & Bedner, 2012). A very effective and energy independent mechanism of glial K$^+$ clearance describes the spatial buffering hypothesis, according to which excessive extracellular K$^+$ is taken up by astrocytes at sides of high neuronal activity, and then redistributed through the astrocytic gap junction-coupled network to be released at distant regions of lower [K$^+$]o. Here, uptake and release of K$^+$ occur passively, via diffusion through Kir4.1 channels (Kofuji & Newman, 2004; Orkand et al., 1966; Walz, 2000).

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### 3.7 Ca$^{2+}$ signaling and gliotransmission in human astrocytes

Intracellular [Ca$^{2+}$] elevations in astrocytes that propagate through the astroglial network have initially been described in rat hippocampal slices (Porter & McCarthy, 1996) and later also in rat and mouse in vivo (Kuga, Sasaki, Takahara, Matsuki, & Ikegaya, 2011; Kurth-Nelson, Mishra, & Newman, 2009). Intracellular Ca$^{2+}$ waves can occur spontaneously or in response to a variety of stimuli, such as mechanical stimulation or neurotransmitters released from synaptic terminals.
Hence, although astrocytes are electrically nonexcitable, they exhibit a specific form of excitability through fluctuations of [Ca\textsuperscript{2+}], and the intercellular spread of Ca\textsuperscript{2+} waves provides an alternative mechanism for long-distance signaling in the brain that is independent of neurons (Lailouette, De Pittà, & Berry, 2019; Scemes & Giaume, 2006). Increased astrocytic [Ca\textsuperscript{2+}], in turn, induces release of gliotransmitters (such as glutamate, ATP, and D-serine) that modulate synaptic transmission (Bezzi et al., 2004; Bindocci et al., 2017; Halassa et al., 2007; Henneberger et al., 2010).

Ca\textsuperscript{2+} signaling in human astrocytes was first studied in acute slices from the neocortex (Oberheim et al., 2009). The authors showed that Ca\textsuperscript{2+} signals evoked in a single cortical astrocyte by photolysis of caged Ca\textsuperscript{2+} may induce subsequent Ca\textsuperscript{2+} wave propagation. Interestingly, the speed of Ca\textsuperscript{2+} wave propagation was about 5 times faster in human compared to rodent cortex. This study also revealed that [Ca\textsuperscript{2+}], elevations in human astrocytes can be triggered by ATP and glutamate, which is similar to rodent astrocytes (Oberheim et al., 2009). In a later study, Navarrete et al. (2013) reported that human astrocytes in acute hippocampal and cortical slices from TLE patients exhibit spontaneous Ca\textsuperscript{2+} transients that were independent of neuronal activity. Moreover, astrocyte Ca\textsuperscript{2+} elevations could be stimulated by local application of glutamate, cannabinoid, and purinergic receptor agonists as well as by electrical stimulation, indicating that they are induced by synaptic activity (Navarrete et al., 2013). Intriguingly, the authors could also show that astrocyte [Ca\textsuperscript{2+}], transients stimulated by local ATP application increased the frequency of NMDA-mediated currents in both cortical and hippocampal neurons, suggesting that human astrocytes, like their rodent counterparts, are able to modulate synaptic activity through Ca\textsuperscript{2+}-dependent release of glutamate. These data provide evidence for the presence of gliotransmission and bidirectional neuron-astrocyte signaling in the human cortex and hippocampus, and it is, therefore, reasonable to assume that the concept of the “tripartite synapse” proposed for rodents applies also to the human brain (Navarrete et al., 2013).

4 | CONCLUSIONS

The important role of glial cells for proper brain signaling is increasingly recognized, and evidence emerges suggesting that dysfunctional glial cells are key players in the etiology of neurological diseases (Parpura et al., 2012; Seifert et al., 2006). As medical research relies on animal models, knowledge about species differences in the properties of glial cells is vital to predict the translatability of animal data to humans. Rodents are the most widely used experimental model organisms to investigate human diseases. Surprisingly, careful comparison reveals that astrocytes and NG2 glia in rodents and human share many similar properties.

Morphological analyses revealed a higher glia-to-neuron ratio in the human versus rodent neocortex (1.6 vs. 0.3; Verkhratsky & Nedergaard, 2016). Human cortical astrocytes are larger, contact more synapses, and are more diverse than their rodent counterparts (Colombo et al., 1995; Oberheim et al., 2009). Intriguingly, results available so far demonstrate that the functional properties of astrocytes and NG2 glial cells are amazingly similar between rodents and humans. However, the current data situation must be interpreted with caution. First, functional data are only available from two human brain regions, neocortex and hippocampus, and it is unknown whether this similarity also applies to other areas of the CNS. Notably, rodent studies have revealed a high degree of functional heterogeneity among astrocytes, both within a given and across different brain regions. Second, in neurosurgically resected “control-like” human brain tissue, even if lacking obvious anatomical alterations, medication may have affected cellular properties. Third, reliable analysis of astrocyte function and its interactions are principally hampered by the very low electrical compactness of these cells and their fine distant processes, which fall below current optical resolution (Ma, Xu, Wang, Enyeart, & Zhou, 2014; Seifert et al., 2009). This limitation applies to both rodent and human astrocytes and hampers precise biophysical analyses and comparison. Finally, it should be considered that in many studies, different experimental conditions were chosen (K+ concentrations in the solutions used, temperature, age of animals, and so on), which limits the comparability of published data. Previous reviews even compared properties of human NG2 glia with rodent astrocytes, leading to wrong conclusions as to apparent species-dependent differences. Despite these limitations, it can be concluded that based on the data currently available, human and rodent glial cells share many functional properties, providing justification for using mouse or rat models to investigate causes of human brain diseases.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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REFERENCES

Araque, A., Carmignoto, G., Haydon, P. G., Ollet, S. H. R., Robitaille, R., & Volterra, A. (2014). Gliotransmitters travel in time and space. Neuron, 81(4), 728–739. https://doi.org/10.1016/j.neuron.2014.02.007
Araque, A., Parpura, V., Sanzgiri, R. P., & Haydon, P. G. (1999). Tripartite synapses: Glia, the unacknowledged partner. Trends in Neurosciences, 22(5), 208–215 https://doi.org/10.1016/S0166-2236(99)01349-6.
Aronica, E., Gorter, J. A., Jansen, G. H., Leenstra, S., Yankaya, B., & Troost, D. (2001). Expression of connexin 43 and connexin 32 gap-
hippocampal slice. Hippocampus, 4(1), 19–35. https://doi.org/10.1002/hipo.450040105

Steinhäuser, C., Kressin, K., Kuprijanova, E., Weber, M., & Seifert, G. (1994). Properties of voltage-activated Na+ and K+ currents in mouse hippocampal glial cells in situ and after acute isolation from tissue slices. Pflügers Archiv, 428(5), 610–620. https://doi.org/10.1007/BF00374585

Steinhäuser, C., Seifert, G., & Bedner, P. (2012). Astrocyte dysfunction in temporal lobe epilepsy: K+ channels and gap junction coupling. GLIA, 60(8), 1192–1202. https://doi.org/10.1002/glia.22313

Tang, X., Taniguchi, K., & Kofuji, P. (2009). Heterogeneity of Kir4.1 channel expression in glia revealed by mouse transgenesis. GLIA, 57(16), 1706–1715. https://doi.org/10.1002/glia.20882

Trotter, J., Karram, K., & Nishiyama, A. (2010). NG2 cells: Properties, progeny and origin. Brain Research Reviews, 63(1), 72–82. https://doi.org/10.1016/j.brainresrev.2009.12.006

Steinhäuser, C., Kressin, K., & Nishiyama, A. (2009). NG2 cells: Properties, progeny and origin. Brain Research Reviews, 63(1), 72–82. https://doi.org/10.1016/j.brainresrev.2009.12.006

Vasile, F., Dossi, E., & Rouach, N. (2017). Human astrocytes: Structure and functions in the healthy brain. Brain Structure & Function, 222(5), 2017–2029. https://doi.org/10.1007/s00429-017-1383-5

Verkhratsky, A., & Nedergaard, M. (2018). Physiology of Astroglia. Physiological Reviews, 98(1), 239–389. https://doi.org/10.1152/physrev.00042.2016

Verkhratsky, A., & Parpura, V. (2015). Physiology of Astroglia: Channels, receptors, transporters, ion signaling and gliotransmission. Colloquium Series on Neuroglia in Biology and Medicine: From Physiology to Disease, 2 (2), 1–172. https://doi.org/10.4199/C00123ED1V01Y201501NGL004

Verkhratsky, A., & Nedergaard, M. (2016). The homeostatic astroglia emerges from evolutionary specialization of neural cells. Philosophical Transactions of the Royal Society B: Biological Sciences, 371(1700), 20150428. https://doi.org/10.1098/rstb.2015.0428

Virkow, R. (1859). Die Cellulopathologie in ihrer Begründung auf physiologische und pathologische Gewebelehre: zwanzig Vorlesungen, gehalten während der Monate Februar, März und April 1858 im pathologischen Institute zu Berlin. Medicus-Verlag.

Virkow, R. (1856). Gesammelte Abhandlungen zur Wissenschaftlichen Medizin. Frankfurt, Germany: Meidinger Sohn & Comp Available from http://archive.org/details/b21462161.

Wallraff, A., Köhling, R., Heinemann, U., Theis, M., Willecke, K., & Steinhäuser, C. (2006). The impact of astrocytic gap junctional coupling on potassium buffering in the hippocampus. Journal of Neuroscience, 26(20), 5438–5447. https://doi.org/10.1523/JNEUROSCI.0037-06.2006

Wallraff, A., Odermatt, B., Willecke, K., & Steinhäuser, C. (2004). Distinct types of astrogial cells in the hippocampus differ in gap junction coupling. GLIA, 48(1), 36–43. https://doi.org/10.1002/glia.20040

Walz, W. (2000). Role of astrocytes in the clearance of excess extracellular potassium. Neurochemistry International, 36(4), 291–300. https://doi.org/10.1016/S0197-0186(99)00137-0

Wennergren, A., Hellsten, J., Ekdh, C. T., & Tingström, A. (2003). Electrocortical seizures induce proliferation of NG2-expressing glial cells in adult rat hippocampus. Biological Psychiatry, 54(10), 1015–1024. https://doi.org/10.1016/j.biopsych.2004.03.069

Wilson, H. C., Scolding, N. J., & Raine, C. S. (2004). Co-expression of PDGF α receptor and NG2 by oligodendrocyte precursors in human CNS and multiple sclerosis lesions. Journal of Neuroimmunology, 176(1), 162–173. https://doi.org/10.1016/j.jneuroim.2006.04.014

Xie, M., Lynch, D. T., Schools, G. P., Feustel, P. J., Kimelberg, H. K., & Zhou, M. (2007). Sodium channel currents in rat hippocampal NG2 glia: Characterization and contribution to resting membrane potential. Neuroscience, 150(4), 853–862. https://doi.org/10.1016/j.neuroscience.2007.09.057

Zhu, X., Bergles, D. E., & Nishiyama, A. (2008). NG2 cells generate both oligodendrocytes and gray matter astrocytes. Development, 135(1), 145–157. https://doi.org/10.1242/dev.004895

Zhu, X., Hill, R. A., Dietrich, D., Komitova, M., Suzuki, R., & Nishiyama, A. (2011). Age-dependent fate and lineage restriction of single NG2 cells. Development (Cambridge, England), 138(4), 745–753. https://doi.org/10.1242/dev.047951

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