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Physiological characteristics of NG2-expressing glial cells

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Abstract

Antibodies against the chondroitin sulfate proteoglycan NG2 label a subpopulation of glial cells within the CNS, which have a small cell body and thin radiating processes. Physiological recordings from these small cells in acute brain slices have revealed that they possess unique properties, suggesting that they may comprise a class of glial cells distinct from astrocytes, oligodendrocytes, or microglia. NG2-expressing glial cells (abbreviated as "NG2 cells" here) have a moderate input resistance and are not dye- or tracer-coupled to adjacent cells. They express voltage-gated Na^+, K^+ and Ca^{2+} conductances, though they do not exhibit regenerative Na^+ or Ca^{2+} action potentials due to the much larger K^+ conductances present. In addition to voltage-gated conductances, they express receptors for various neurotransmitters. In the hippocampus, AMPA and $GABA_A$ receptors on these cells are activated by release of transmitter from neurons at defined synaptic junctions that are formed with CA3 pyramidal neurons and GABAergic interneurons. These rapid forms of neuron-glial communication may regulate the proliferation rate of NG2 cells or their development into mature oligodendrocytes. These depolarizing inputs may also trigger the release of neuroactive substances from NG2 cells, providing feedback regulation of signaling at neuronal synapses. Although the presence of Ca^{2+} permeable AMPA receptors provides a pathway to link neuronal activity to Ca^{2+} dependent processes within the NG2 cells, these receptors also put these cells at risk for glutamate-associated excitotoxicity. This vulnerability to the sustained elevation of glutamate may underlie ischemic induced damage to white matter tracts and contribute to cerebral palsy in premature infants.

Introduction

Glial cells comprise a diverse group of non-neuronal cells that are specialized for equally diverse functions in the CNS; they remove the ions and neurotransmitters released during neural activity, they provide the insulating sheets of myelin that allow rapid propagation of this activity over long distances, and they provide the energy necessary to support this activity. There is increasing evidence that in addition to these largely supportive functions, certain types of glial cells may influence signal transduction at synapses by controlling the amount of neurotransmitter available to bind to receptors (Asztely *et al*., 1997), and by releasing neurotransmitters capable of activating neuronal receptors directly (Kang *et al*., 1998; Parpura & Haydon, 2000; Parri *et al*., 2001). While it is generally accepted that there are three main classes of glial cells (astrocytes, oligodendrocytes, and microglia), anatomical and physiological studies over the past 20 years have provided evidence that some glial cells do not readily fall into this classification scheme. In particular, non-neuronal cells have been described in the hippocampus, cerebellum, brainstem, and the white matter tracts of the corpus callosum and optic nerve, that have properties in common with both neurons and glial cells. These small cells have a stellate shape similar to astrocytes, but are not immunoreactive for glial fibrillary acidic protein (GFAP), do not exhibit dye coupling through gap junctions, do not have a high resting conductance to K^+, and do not express a high density of glutamate transporters, all of which are characteristics of mature gray matter astrocytes. However, unlike neurons, these cells do not have an axon, they are not capable of firing action potentials, and they do not express neuronal markers. As these cells are clearly distinct from oligodendrocytes or microglial cells, and are present both in developing tissue and in the adult brain, it has been suggested that these non-neuronal cells may comprise a distinct class of glial cell (Nishiyama *et al*., 1999).

Cells with this unique combination of properties have been described in various physiological studies as

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"complex cells," "immature astrocytes," "glial precursors," or "oligodendrocyte precursor cells", reflecting a general uncertainty about the identity and function of such cells in the CNS. Recent studies indicate that cells in the hippocampus with these properties exhibit immunoreactivity to the chondroitin sulfate proteoglycan NG2 (mouse AN2 protein) (Bergles *et al*., 2000), a protein that is expressed by cells in the oligodendrocyte lineage (Levine & Nishiyama, 1996; Diers-Fenger *et al*., 2001). For this reason, these cells have been termed oligodendrocyte precursor cells, though it is unclear whether all NG2 cells function as oligodendrocytes progenitors in the mature brain. These NG2 cells share many properties with a group of bipotential glial cells present in cultures of dissociated brain tissue, termed O-2A cells for their ability to give rise to both oligodendrocytes and so-called "Type-2 astrocytes" *in vitro* (Raff *et al*., 1983), supporting the idea that NG2 cells may indeed be oligodendrocyte progenitors at some point during development. This review will describe the physiological properties of NG2 expressing glial cells, as determined from electrophysiological and anatomical studies performed in acute brain slices from rats and mice. The similarities between NG2 cells and cells previously described as complex cells/immature astrocytes/glial precursors suggest that these cells may all correspond to a single class of glial cells. However, as most previous studies did not examine whether a particular cell expressed NG2, it remains possible that a greater diversity of glial cells may be present. While we are still at an early stage in the study of NG2-expressing glial cells, current data indicate that they exhibit a number of interesting and unique properties, suggesting that they play an important role in brain physiology.

Classification of NG2 cells

Glial cells within the CNS have been classified according to their immunoreactivity to a myriad of antibodies against different markers. Immunocytochemical studies indicate that many small stellate cells in the CNS express the NG2 proteoglycan (Fig. 1A) (Levine & Chard, 1987; Nishiyama *et al*., 1997), rather than traditional astrocyte markers (*e.g.* GFAP). This protein has been shown to inhibit axonal growth (Chen *et al*., 2002), suggesting that these cells may be involved in axonal outgrowth, targeting, or perhaps rearrangement of neuronal connectivity. NG2 cells do not exhibit immunoreactivity to antigens such as O4/O1, characteristic of more mature oligodendrocytes progenitors; they also do not exhibit immunoreactivity to myelin basic protein or proteolipid protein (PLP), proteins expressed by mature oligodendrocytes (Levine *et al*., 2001). Nevertheless, recent studies of mice engineered to express the enhanced green fluorescent protein (EGFP) under control of a portion of the PLP promoter revealed that NG2 immunoreactive cells were EGFP positive (Mallon *et al*., 2002). A similar approach has shown that the promoter for 2′-3′-cyclic nucleotide 3′-phosphodiesterase (CNP) is also active in these cells (Belachew *et al*., 2001), supporting the conclusion that these cells share a lineage relationship with oligodendrocytes.

A current controversy surrounds the question of whether the cells exhibiting a complex current-voltage relationship, termed complex cells (or astrocytes), and those that are immunoreactive to NG2 are distinct classes of glia. Unlike astrocytes, neither NG2 cells nor complex cells exhibit immunoreactivity to GFAP, though GFAP mRNA has been described in complex cells using RT-PCR (Zhou *et al*., 2000; Zhou & Kimelberg, 2001), and cells with properties similar to NG2 cells and complex cells express EGFP in GFAP-EGFP transgenic animals (Nolte *et al*., 2001). Such results suggest that complex cells may represent a type of astrocyte that expresses low amounts of GFAP. Complex cells have also been shown to express S100β (Akopian *et al*., 1997; Seifert *et al*., 1997a), a Ca^{2+} binding protein/cytokine that is highly expressed by astrocytes, supporting the conclusion that complex cells are closely related to astrocytes. However, recent studies indicate that S100β is not an exclusive marker for astroglial cells, as CNPase expressing cells in the hippocampus also express this protein (Ogata & Kosaka, 2002), and both astrocytes and NG2 cells express EGFP in transgenic mice engineered to express EGFP under control of the S100β promoter (Lubischer *et al*., *Soc. Neurosci. Abs.*, 2000) (Fig. 2). The close similarities in morphological, physiological (see below), and biochemical properties between complex cells and NG2 cells suggest that they may represent the same group of cells. Alternatively, it is possible that these cells are distinct, and vary in properties that have not yet been assessed. Nevertheless, these results indicate that the cells which express NG2 share properties with both neurons and glia. Unfortunately, such studies have provided few clues about the function(s) of these enigmatic cells.

Ion channel expression

GENERAL MEMBRANE PROPERTIES

A conspicuous difference between NG2 cells and either astrocytes or oligodendrocytes is their higher membrane resistance. NG2 cells *in situ* exhibit an input resistance of >200 MΩ (range 200–400 MΩ), when measured at the resting potential (between -70 and -90 mV) with a K^+-based internal solution in whole-cell recordings (Fig. 3 A1 and 2). Their membrane resistance is closer to that exhibited by pyramidal neurons in the hippocampus than to astrocytes which have an input resistance of 10 MΩ or less depending on the extent of gap junctional coupling and level of expression of a leak K^+ conductance (Fig. 3B1 and 2). Despite the higher membrane resistance of NG2 cells, their high

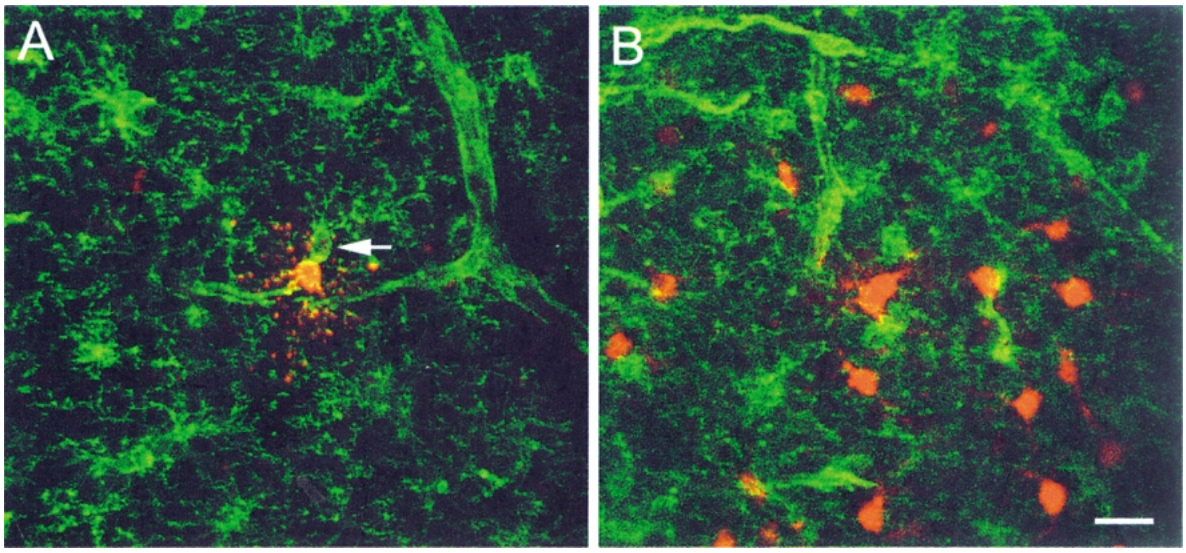

Fig. 1. Comparison of tracer coupling between NG2 cells and astrocytes in the hippocampus. A, A putative OPC in the stratum radiatum region of area CA1 of the hippocampus was loaded with biocytin (0.15%) through the whole-cell electrode. The biocytin was detected with Cy5-streptavidin (*red*) and NG2 immunoreactivity was detected using a rabbit polyclonal antibody raised against NG2 (courtesy of Dr. Bill Stallcup) and Cy2 conjugated goat anti-rabbit (*green*). Despite the close apposition between the recorded cell and another NG2 immunoreactive cell (*arrow*), no tracer coupling was observed between them. B, A whole-cell recording from an astrocyte under similar conditions results in diffusion of the biocytin to numerous adjacent cells. These cells do not exhibit NG2 immunoreactivity. Scale bar = 20 μm. A and B from a P14 rat.

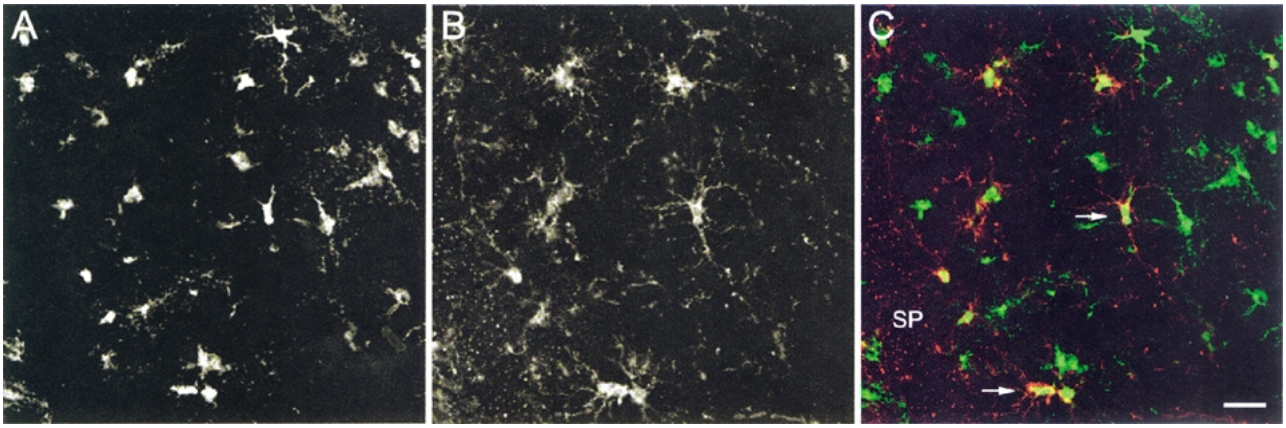

Fig. 2. Expression of EGFP by NG2 cells in S100β-egfp transgenic mice. A, EGFP expression in the CA1 region of the hippocampus from a transgenic mouse expressing EGFP under control of the S100β promoter (Lubischer *et al.*, *Soc. Neurosci. Abs.*, 2000). B, NG2 immunoreactivity from the same region of the slice shown in A. C, Composite image of both EGFP (*green*) and NG2 (*red*) from this region of the hippocampus. Two examples of NG2-immunoreactive cells that also expressed EGFP are indicated by the *arrows*. NG2 immunoreactivity was detected using a rabbit polyclonal antibody raised against NG2 (courtesy of Dr. Bill Stallcup) and Cy3 conjugated donkey anti-rabbit secondary antibody. Scale bar = 20 μm.

resting membrane potential indicates that their membranes are largely permeable to K^+ at rest (see below).

NG2 cells in the hippocampus do not exhibit dye coupling; introduction of neurobiotin or small fluorescent tracer molecules such as Alexa 488 though the recording electrode, does not result in the transfer of these molecules to adjacent cells (Akopian *et al.*, 1996; Bergles *et al.*, 2000) (Fig. 1A), unlike astrocytes, which exhibit extensive dye coupling (Fig. 1B). Although these data indicate that NG2 cells are not extensively coupled via gap junctions, it is possible that small numbers of gap junctions could be present to allow significant electrical coupling. Electrical coupling is often not detected by tracer coupling, as shown recently for cortical interneurons (Gibson *et al.*, 1999). Paired recordings from NG2 cells and other cells have not yet been performed to address this possibility.

K^+ CHANNELS

Injection of positive current into NG2 cells elicits nonlinear, time-dependent changes in membrane potential

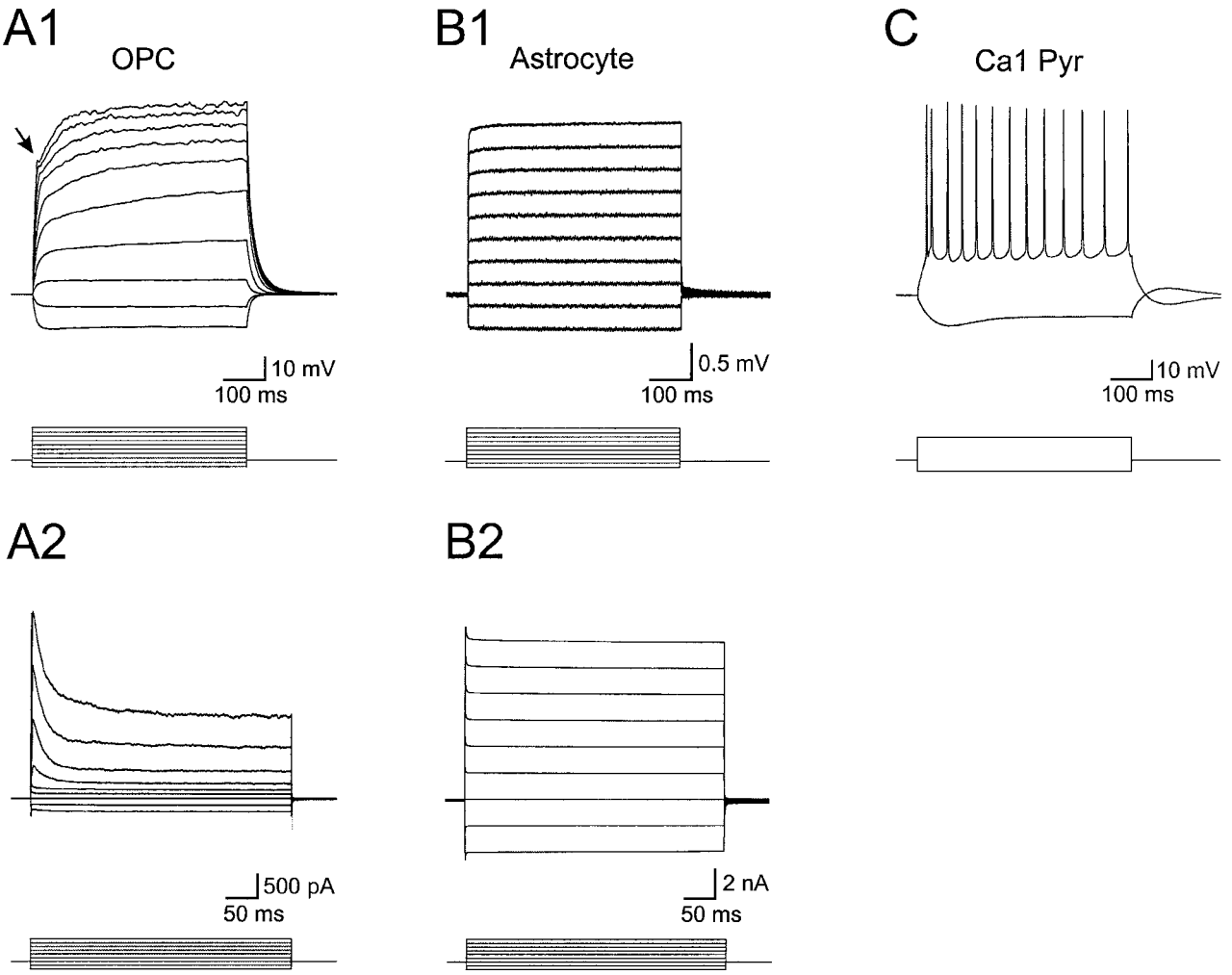

Fig. 3. Membrane properties of NG2-expressing glial cells, astrocytes and CA1 pyramidal neurons. A1, Whole-cell current-clamp recording from a hippocampal OPC. Negative current injection resulted in a slight sag in the membrane potential back to the resting level, while positive current injection elicited a complex membrane response due to activation of Na^+, Ca^{2+} and K^+channels. A2, Whole-cell voltage clamp recording from the same cells shown in A1. Hyperpolarizing voltdage steps reveal the presence of inward K^+ currents and a small I_h current, while depolarizing voltage steps reveal the presence of prominent A-type and sustained delayed-rectifier type K^+ currents. Activation of Na^+ channels results in a small inflection on the rising phase of the depolarization (*arrow*). Steps: −60 pA to 300 pA, 40 pA increments from −90 mV in A1, and −130 mV to 20 mV, 20 mV increments in A2 from −90 mV. B, Whole-cell current-clamp recording from an astrocyte located in stratum radiatum area CA1. The same negative and positive current injection used in A1 elicited only small deviations of the membrane potential due to the high resting conductance. $Vm = -90$ mV. B2, Voltage-clamp responses to hyperpolarizing and depolarizing voltage steps reveal the linear behavior of the astrocyte membrane. C, Whole-cell current-clamp recording from a CA1 pyramidal neuron. Negative current injection (−60 pA) from the resting potential (−67 mV) results in a sag of the membrane potential back to the resting level, indicative of activation of an I_h current, while positive current injection (120 pA) results in the triggering of action potentials. A Kmethanesulfonate based internal solution containing 0.2 mM EGTA was used for all recordings.

(Fig. 3A1), unlike the passive behavior of astrocyte membranes (Fig. 3B1). The predominant voltage-gated conductances responsible for these changes in membrane potential are carried by K^+. Depolarizing voltage steps from the resting potential elicit both rapidly inactivating and sustained K^+ currents (Fig. 3A2). These currents consist of conventional "A-type" (I_A) and "delayed rectifier" type (I_K) K^+ currents that are antagonized by 4-aminopyridine and tetraethylammonium, respectively (D. Bergles, unpublished results). Complex cells also express such currents (Kressin *et al.*, 1995; Akiopan *et al.*, 1997), as do the majority of GFAP immunoreactive astrocytes (Tse *et al.*, 1992; Bordey & Sontheimer, 1997; D'Ambrosio *et al.*, 1998), though in astrocytes they are often overshadowed by large leak K^+ currents and extensive gap junctional coupling (Fig. 1B and Fig. 3B2). In contrast, oligodendrocytes exhibit voltage-independent currents that decay during

positive current injection, a phenomenon that results from an accumulation of K^+ in the extracellular space, a subsequent shift in the K^+ reversal potential, and reduction in driving force for K^+ (Steinhauser *et al*., 1992). Microglial cells exhibit a high input resistance and only inward rectifying K^+ currents (Boucsein *et al*., 2000). Cultured O-2A cells have been shown to express predominately mRNA for Kv 1.2, 1.5, and 1.6, though Kv 1.3 and 1.4 were detected in some cells (Schmidt *et al*., 1999), providing clues to the possible molecular identity of these K^+ channels. In addition to these outward K^+ currents, NG2 cells also exhibit inward K^+ currents to a variable degree (Fig. 3A2). These currents are likely to result from the activity of both ATP-sensitive K^+ channels (K_{ATP}), and inwardly rectifying K^+ channels (Kir). Kir channels stabilize the membrane potential near the K^+ equilibrium potential, and may be involved in the accumulation, buffering, or siphoning of K^+ released during neuronal activity (Reimann & Ashcroft, 1999). K^+ channels gated by ATP are composed of Kir 6.x and SUR subunits, and provide a means to link cell metabolism to excitability and K^+ flux (Reimann & Ashcroft, 1999). K_{ATP} currents have been described in glial cells located in the CA1 stratum radiatum region of the hippocampus that had properties similar to NG2 cells (Zawar *et al*., 1999). In addition, complex cells have been shown to exhibit similar inwardly rectifying K^+ channels (Steinhauser *et al*., 1992), and single-cell RT-PCR from hippocampal complex cells revealed the presence of mRNA for Kir subunits 2.1, 2.2, 2.3, and less frequently Kir 4.1 (Schroder *et al*., 2002). It is interesting to note that Kir 4.1 has been immunolocalized to some putative NG2 cells (Poopalasundaram *et al*., 2000) and Kir 4.1 knockout animals exhibit a profound disruption of myelination in the spinal cord (Neusch *et al*., 2001), suggesting an important role for this channel in oligodendrocyte maturation or in the maintenance of myelin sheaths. Both transient currents and delayed rectifier currents were previously reported to decrease with age in complex cells, while inward rectifying currents increased (Kressin *et al*., 1995); however, such changes in ion channel expression have not been observed in NG2 cells (Bergles *et al*., 2000).

Na^+ CHANNELS

Like neurons, NG2 cells express voltage-gated Na^+ channels. When K^+ channels are blocked with TEA and Cs^+, depolarization of these cells leads to a rapid transient inward current that is blocked by the Na^+ channel toxin, tetrodotoxin (TTX) (S. Lin & D. Bergles, unpublished results). Under physiological conditions, these Na^+ channels produce a small inflection on the rising phase of membrane potential responses following depolarizing current injection (Fig. 3A1, *arrow*). However, the peak amplitude of the Na^+ current is >10x smaller than that observed in neurons under similar conditions, indicating that they express only a fraction of the Na^+ channels. The small number of these channels and the comparatively large K^+ conductances present prevent NG2 cells from generating action potentials. The presence of Na^+ channels raise the intriguing possibility that NG2 cells might generate slow Na^+ spikes if K^+ channels were subject to inhibition in these cells. Such modulation of K^+ conductances has been described in complex cells following application of glutamate and $GABA_A$ receptor agonists (see below), though it is not yet known if such modulation would elicit a transition to an excitable phenotype, or if such modulation occurs physiologically. O-2A cells in culture and acutely isolated from optic nerve express Na^+ channels that activate over a similar voltage range (Barres *et al*., 1990b). Astrocytes *in situ* (Barres *et al*., 1990a) an *in vitro* culture also express voltage-gated Na^+ channels, though this channel exhibits slower kinetics and more negative voltage dependence of activation and inactivation than Na^+ channels present in NG2 cells and neurons. What might the function of these channels be? The influx of positive charge through the Na^+ channels could boost other depolarizing stimuli allowing activation of other voltage-dependent channels. Alternatively, in the absence of excitability, the Na^+ influx through these channels could regulate the efficiency of Na^+ dependent transporters, the Na^+/K^+ ATPase, or inhibit K^+ channels as described above.

Ca^{2+} CHANNELS

Ca^{2+} channels play a central role in coupling membrane depolarization to changes in cell physiology. Voltage-gated Ca^{2+} channels have been described in complex cells in hippocampal slices (Akopian *et al*., 1996), though the size of Ca^{2+} currents recorded from these cells are quite small compared to those present in neurons. These cells expressed low voltage activated, Cd^{2+}-sensitive currents, and high voltage activated, dihydropyridine and omega-conotoxin GVIA-sensitive Ca^{2+} channels, and depolarization of complex cells from the resting potential to 0 mV for 10 seconds elicited rises in intracellular Ca^{2+} as reported by Fura-2 (Akopian *et al*., 1996). The voltage range over which they begin to open (>20 mV depolarized from resting membrane potential) raises questions about how they might become activated, given that glial cells are thought to be largely quiescent. Recent data indicate that neuronal activity can depolarize NG2 cells (see below), allowing activation of these channels and enhancement of Ca^{2+} influx. The resulting increase in intracellular Ca^{2+} could lead to changes in cell physiology, gene expression, or perhaps release of neuroactive substances. Conversely, Ca^{2+} channels could also help limit depolarization by opening Ca^{2+}-activated K^+ channels.

CONCLUSIONS

The results described above indicate that NG2 cells express a complex repertoire of voltage-gated channels that lead to active membrane properties reminiscent of neurons. However, unlike neurons, these cells are not capable of generating regenerative Na^+ or Ca^{2+} action potentials normally, due to the larger K^+ conductances present. If these K^+ channels were reduced through physiological or pathological activity, a slow form of excitability might be possible. The biophysical properties of NG2 cells contrast with those exhibited by oligodendrocytes and microglia, but the situation is less clear for astrocytes. Although many of these same ion channels are present in astrocytes, the high resting conductance of the astrocyte membrane limits their activation *in situ*. The properties of NG2 expressing cells are remarkably similar to O-2A cells, either maintained in culture or acutely isolated from optic nerve, which express a low density of neuronal type Na^+ currents, A-type K^+ currents, and delayed rectifier K^+ currents, though inwardly rectifying K^+ currents were only observed in cultured O-2A progenitors (Barres *et al*., 1990a). The low resting conductance and the presence of voltage-gated channels indicate that the membrane potential of NG2 cells is likely to change dynamically over a wide range *in vivo*.

Neurotransmitter receptor expression

GLUTAMATE RECEPTORS

Early studies of the expression of non-NMDA receptor subunits in the brain revealed that a subset of non-neuronal cells with morphological features similar to astrocytes expressed the GluR4 subunit (Ong & Garey, 1996). Whole-cell recordings from complex cells in hippocampal slices (Steinhauser *et al*., 1994), glial precursor cells in the corpus callosum (Berger, 1995), and in O-2A cells acutely isolated from optic nerve (Barres *et al*., 1990a) revealed that glutamate or kainate, agonists at AMPA/kainate receptors, elicited inward currents in these cells. Furthermore, whole-mount preparations of optic nerve revealed that O-2A cells have the ability to accumulate cobalt in the presence of quisqualate, an AMPA/kainate receptor agonist. This accumulation was blocked by CNQX, an AMPA/kainate receptor antagonist (Fulton *et al*., 1992), suggesting that quisqualate opens AMPA receptors in these cells and allows cobalt to enter the cell. Subsequent pharmacological and molecular biological studies involving single cell RT-PCR have revealed that complex cells isolated from the hippocampus express the AMPA receptor subunits GluR1-4 (Seifert *et al*., 1997a; Seifert *et al*., 1997b), with GluR2 and GluR4 being the most common. Pharmacological analysis indicates that NG2 cells *in situ* express primarily AMPA receptors, as glutamate evoked responses were blocked by GYKI 53655 (Bergles *et al*., 2000), a compound that is selective for AMPA receptors (Dingledine *et al*., 1999), and were dramatically enhanced by cyclothiazide (Fig. 4A), a compound that inhibits desensitization of AMPA receptors. The ability of AMPA/kainate receptor antagonists to completely block responses to glutamate indicates that NMDA receptors are not expressed by NG2 cells. These results are similar to the pattern of glutamate receptor expression in O-2A cells, which express both AMPA and kainate receptors, but not NMDA receptors (Barres *et al*., 1990a; Holzwarth *et al*., 1994; Patneau *et al*., 1994).

In addition to providing a route for the influx of positive charge, AMPA receptors composed of subunits lacking the edited GluR2(B) subunit allow Ca^{2+} to flow into the cell when activated. Although GluR2 subunits are expressed by acutely isolated complex cells (Seifert *et al*., 1997a), AMPA responses in these cells exhibit a PCa^{2+}/PCs^+ ratio of about 0.3 (Seifert *et al*., 1997a; Seifert *et al*., 1997b) indicating that they have a substantial permeability to Ca^{2+}, and that a portion of the AMPA receptors expressed by NG2 cells lack the GluR2 subunit. These data are consistent with the sensitivity of AMPA receptor currents in NG2 cells to inhibition by spermine (Fig. 4C) (Bergles *et al*., 2000), a polyamine that blocks outward current through GluR2-lacking AMPA receptors. Although the Ca^{2+} permeability of glutamate receptors was reported to decrease during first three postnatal weeks in glial precursors in the dentate gyrus (Backus & Berger, 1995), the Ca^{2+} permeability of acutely isolated complex cells in the CA1 region was constant from P5-P35 (Seifert *et al*., 1997b).

To date, there have been no reports of metabotropic glutamate (mGluR) expression by NG2 cells.

GABA RECEPTORS

Previous studies have shown that application of GABA or the $GABA_A$-selective agonist muscimol, elicited inward currents in glial precursors in corpus callosum slices (Berger, 1995) in complex cells in hippocampal slices (Steinhauser *et al*., 1994), and in O-2A cells in culture (Williamson *et al*., 1998). We have shown that NG2 cells in the hippocampus also express $GABA_A$ receptors: puffer application of muscimol elicits transient inward currents in Cl^- loaded cells that are inhibited by bicuculline, picrotoxin, or SR-95531 (gabazine) (Fig. 5A) (Lin & Bergles, *Soc. Neurosci. Abs.* 2001). Furthermore, in outside-out patches isolated from these cells, brief application of GABA elicited rapidly activating inward currents that were completely blocked by SR-95531, demonstrating the expression of functional $GABA_A$ receptors by NG2 cells (Lin & Bergles, *Soc. Neurosci. Abs.* 2001). These results indicate that NG2 cells express functional $GABA_A$ receptors *in situ*. O-2A cells have also been shown to express $GABA_A$ receptors (von Blankenfeld *et al*., 1991; Williamson *et al*., 1998), which exhibit a low affinity for GABA and slow

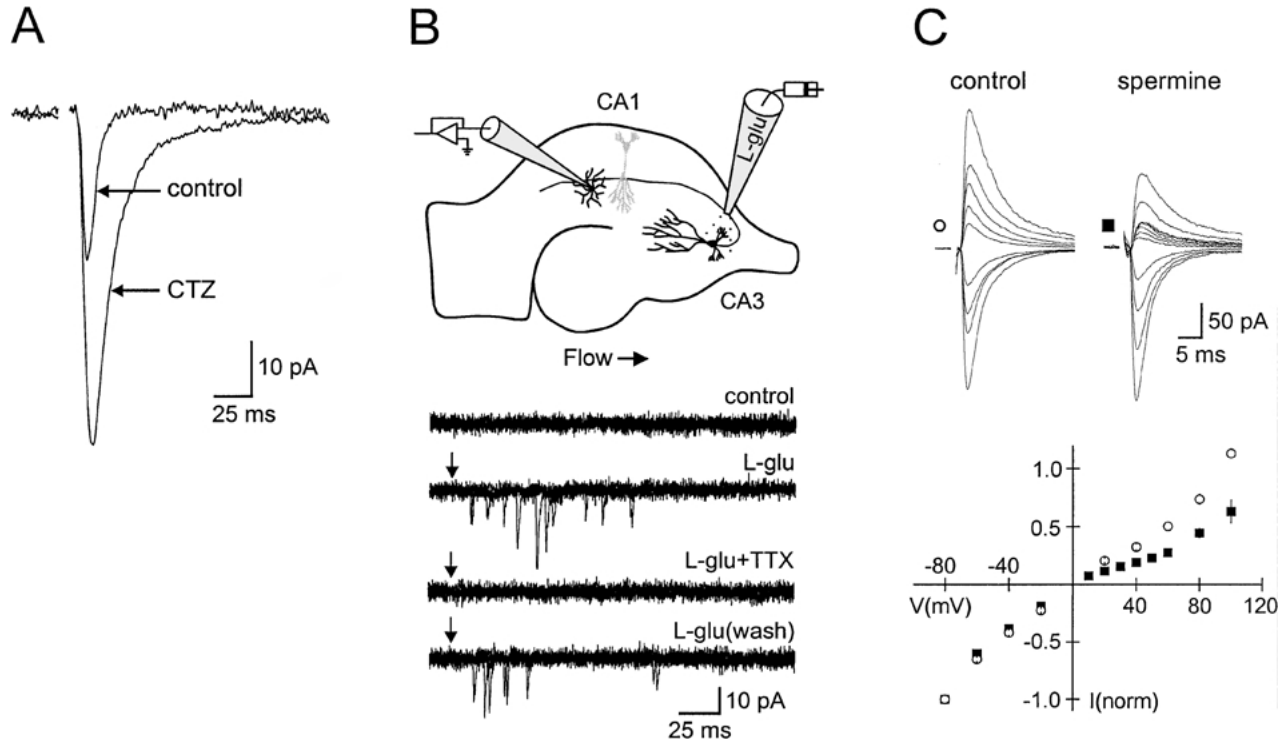

Fig. 4. Characteristics of glutamatergic synaptic currents in hippocampal NG2 cells. A, Stimulation of Schaffer collateral-commissural axons in the stratum radiatum region elicited an inward current in an OPC voltage-clamped at −80 mV. Bath application of cyclothiazide (CTZ, 200 μM) increased the amplitude and slowed the decay of the evoked response. B, Puffer application of L-glutamate (200 μM) to area CA3 elicited bursts of excitatory postsynaptic currents in an OPC located in the stratum radiatum region of area CA1. These L-glutamate-evoked responses were reversibly blocked by tetrodotoxin (TTX, 1 μM). C, Addition of spermine (25 μM) to the intracellular solution decreased the amplitude of outward AMPA receptor-mediated currents (*top traces*), and enhanced the inward rectification in the current-to-voltage relationship (*below graph*), indicating the contribution of Ca^{2+} permeable AMPA receptors. Reprinted with permission from Bergles *et al*. (2000).

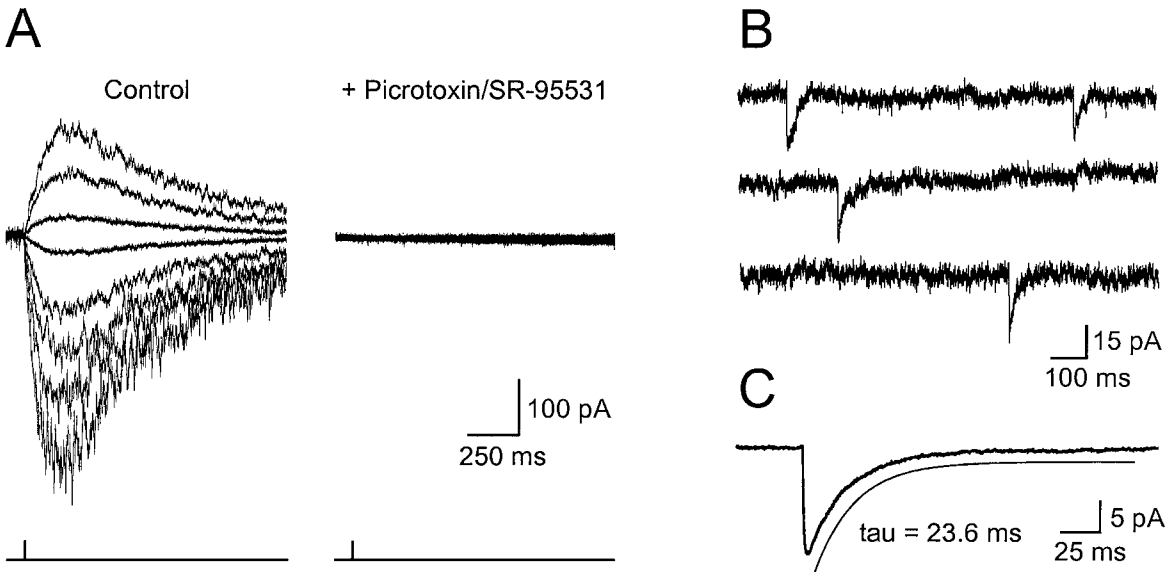

Fig. 5. Characteristics of $GABA_A$ receptor-mediated currents in hippocampal NG2 cells. A, Brief (7 ms) application of the $GABA_A$ receptor selective agonist muscimol (10 mM) (duration shown below) elicited currents in an OPC that reversed close to 0 mV, the predicted reversal potential for Cl^- (*Vm*: −105, −65, −45, −25, −5, 5, 15, 25). These currents were completely blocked by the $GABA_A$ receptor antagonists picrotoxin (100 μM) and SR-95531 (gabazine) (5 μM). B, With an internal solution containing 130 mM Cl^-, whole-cell recordings from NG2 cells revealed the occurrence of spontaneous $GABA_A$ receptor-mediated currents. ($Vm = -80$ mV). C, Average time course of 189 events from the cell in B. These currents decayed with a single exponential fit (*solid line*) that was ∼2× slower than $GABA_A$ receptor-mediated currents in CA1 pyramidal neurons.

activation kinetics. RT-PCR of cultures enriched with O-2A cells indicated that they do not express mRNA for α1, α6, or δ subunits (Williamson *et al*., 1998), but the subunit composition of these receptors has not yet been investigated further. It is not yet known if these cells express $GABA_B$ receptors.

OTHER NEUROTRANSMITTER RECEPTORS

Little is known about the expression of receptors for other neurotransmitters in NG2 cells. However, O-2A cells in culture have been shown to express α and β adrenergic receptors (Ghiani *et al*., 1999), both muscarinic and nicotinic acetylcholine receptors (Rogers *et al*., 2001), and receptors to substance P (Marriott & Wilkin, 1993), among others. These data raise the possibility that NG2 cells may express receptors for many more neurotransmitters, but further studies will be necessary to determine the compliment of receptors expressed by NG2 cells *in situ.*

Neuron-NG2 cell signaling at synapses

The expression of neurotransmitter receptors by NG2 cells raises an important question of how these receptors become activated *in situ*. Neurotransmitters are released from neurons at synapses through fusion of neurotransmitter loaded vesicles with the presynaptic membrane at active zones; transmitter then reaches glial cells primarily following diffusion out of the synaptic cleft (Porter & McCarthy, 1996; Bergles *et al*., 1997; Bergles & Jahr, 1997; Clark & Barbour, 1997). Non-vesicular release of transmitter by reversed transport may also occur during conditions of intense neuronal activity or pathological conditions such as ischemia. However, recent data indicate that glutamate and GABA are released directly onto NG2 cells receptors at neuron-NG2 cell synapses (Bergles *et al*., 2000; Lin & Bergles, *Soc. Neurosci. Abs.*, 2001), countering current dogma that synaptic junctions are only formed between neurons. Stimulation of excitatory afferents in hippocampal slices prepared from two week-old or adult rats elicited fast inward currents in NG2 cells voltage-clamped at their resting potential, these responses were also visible as transient depolarizations of 2–10 mV in current-clamp recordings. These evoked responses were blocked by AMPA receptor antagonists NBQX and GYKI-52466, and were enhanced by cyclothiazide (Fig. 4A). In contrast to recordings from astroglial cells, miniature excitatory postsynaptic currents (mEPSCs) resulting from the fusion of individual synaptic vesicles with the presynaptic membrane were visible in recordings from NG2 cells. These mEPSCs had an average rise time of 250 μs and they decayed in less than 1 ms (@ 22–24°C), exhibiting faster kinetics than mEPSCs recorded from pyramidal neurons. The rapid kinetics of OPC AMPA receptor responses suggest that the concentration profile of glutamate seen by these receptors is very different than that experienced by AMPA receptors on astroglial cells (Bergles *et al*., 1997; Clark & Barbour, 1997). The concentration of glutamate necessary to elicit AMPA receptor-mediated currents with these rapid kinetics was estimated by measuring the concentration-dependence of the kinetics of these receptors in outside-out patches that were removed from NG2 cells in hippocampal slices. Using fast flow techniques that allow rapid solution exchange at the patch, it was determined that a concentration of $\sim$3 mM glutamate was necessary to elicit AMPA receptor currents that had a rise time of $<$250 μs at room temperature. These results indicate that the mEPSCs recorded from NG2 cells are produced by a high concentration of glutamate, similar to that which is achieved in the synaptic cleft following fusion of a transmitter filled vesicle with the presynaptic membrane (Clements *et al*., 1992). These physiological data have led us to conclude that AMPA receptor currents in NG2 cells arise from direct synapses rather than by spillout of glutamate from nearby neuronal synapses. This conclusion is further supported by the high variance of evoked responses (Bergles *et al*., 2000) and the small ($\sim 2\times$) increase in the amplitude of evoked responses produced by cyclothiazide (Fig. 4A), a compound that increases the apparent affinity of AMPA receptors for glutamate (Dzubay & Jahr, 1999).

The structure of these neuron-NG2 cell synaptic junctions was determined using transmission electron microscopy of thin sections prepared from slices containing physiologically characterized NG2 cells that had been filled with biocytin through the recording electrode. These micrographs revealed that axonal boutons containing synaptic vesicles formed defined junctions with the processes of NG2 cells. However, NG2 cell junctions differed from excitatory synapses on neurons—they were smaller, had fewer vesicles, and lacked well-defined post-synaptic densities. Both physiological and morphological data support the conclusion that these boutons arise from CA3 pyramidal neurons: (1) individual boutons were observed that formed synapses both with an NG2 cell process and a dendritic spine of a pyramidal neuron in the stratum radiatum region, where axons from CA3 pyramidal neurons terminate, (2) evoked responses exhibited robust paired-pulse facilitation similar to EPSCs in CA1 pyramidal neurons, (3) evoked EPSCs in NG2 cells were enhanced by α1-adenosine receptors similar to evoked EPSCs in CA1 pyramidal neurons, and (4) pressure application of glutamate to the CA3 region elicited bursts of EPSCs in NG2 cells located in the CA1 region of the same slice that were blocked by TTX (Fig. 4B), indicating that they were dependent on the conduction of action potentials from CA3. As the only glutamatergic neurons in the CA3 region are the pyramidal neurons, these results suggest that the Schaffer collateral-commissural

fibers form synapses both with CA1 pyramidal neurons and NG2 cells. AMPA-receptor dependent EPSCs were also visible in NG2 cells in slices prepared from adult animals, indicating that these neuro-glial synapses persist throughout life and are not transient developmental phenomena.

The current-to-voltage relationship of evoked responses recorded from NG2 cells in P14 slices, measured by eliciting synaptic responses when holding the membrane of NG2 cells at positive and negative potentials, exhibited prominent inward rectification when the polyamine spermine was added to the intracellular solution in slices prepared from two-week old animals (Fig. 4C). Currents mediated by AMPA receptors lacking the GluR2 subunit exhibit a similar rectification due to block of the outward current by internal polyamines (Dingledine *et al*., 1999). This enhancement of inward rectification of the NG2 cell response indicated that receptors underlying the synaptic currents were permeable to Ca^{2+}. These neuro-glial synapses thus provide a mechanism for adjusting the physiology of NG2 cells to changes in neuronal activity via Ca^{2+}-dependent signaling pathways.

Are other neurotransmitter receptors on NG2 cells activated by a similar mechanism? To address this question we looked at whether spontaneous $GABA_A$ receptor-mediated currents could be recorded from NG2 cells. When NG2 cells were loaded with Cl^- through the whole-cell electrode to enhance the size of currents flowing through the $GABA_A$ anion channel, spontaneous inward currents were observed that had a much slower decay (Fig. 5B and C). Many of these currents were quantal, as they persisted in TTX, and they were mediated by $GABA_A$ receptors as they were blocked by SR-95531 or picrotoxin. We believe that these responses result from direct synapses formed between interneurons and NG2 cells, because of their rapid rise time, their relative insensitivity to agents that increase the affinity of $GABA_A$ receptors (*e.g*. diazepam) or agents that block GABA reuptake (SKF-8976A) (Lin & Bergles, *Soc. Neurosci. Abs*., 2001). These data suggest that local circuit interneurons form synapses with NG2 cells in the hippocampus. We do not yet know the structure of these synapses, or their density on NG2 cells.

Consequences of receptor activation

OPC PROLIFERATION AND OLIGODENDROCYTE DEVELOPMENT

In vitro studies suggest that there is a link between neurotransmitter receptor activation and the proliferation and differentiation of NG2 cells. AMPA/kainate receptors are primarily permeable to Na^+ (Dingledine *et al*., 1999), and glutamate-stimulated influx of Na^+ through these receptors has been shown to inhibit K^+ currents (both delayed rectifier and inward rectifier) in O-2A cells (Gallo *et al*., 1996; Knutson *et al*., 1997) and in complex cells in brain slices (Borges & Kettenmann, 1995; Schroder *et al*., 2002). Numerous studies indicate that K^+ channel modulation plays an important role in cell proliferation. This reduction of K^+ currents may represent a mechanism by which glutamate receptors regulate the development of NG2 cells. Indeed, prolonged exposure of O-2A progenitors to glutamate inhibited their proliferation and prevented their maturation into oligodendrocytes (Liu & Almazan, 1995; Gallo *et al*., 1996). Similarly, exposure of oligodendrocyte progenitors in slice cultures to AMPA or kainate reduced thier proliferation, while DNQX, an AMPA/kainate receptor antagonist, increased their proliferation; these effects were also seen with tetraethylammonium, a K^+ channel blocker, but not with a $GABA_A$ antagonist or a muscarinic acetylcholine receptor agonist (Yuan *et al*., 1998; Ghiani *et al*., 1999). An important consideration is that the experiments measuring glutamate-dependent K^+ channel inhibition were performed in whole-cell recordings in which the endogenous Ca^{2+} buffering of the cell was augmented by exogenous buffers. Studies of glial precursor cells in the hilus with perforated-patch recordings that maintain endogenous buffering, indicate that kainate increased the membrane conductance to K^+, presumably as a result of direct Ca^{2+} influx through the receptor and subsequent activation of Ca^{2+}-dependent K^+ channels (Backus *et al*., 1998).

Although *in vitro* studies point to a dominant role for glutamate in regulating progenitor cell proliferation, GABA may also be important. Experiments performed on glial precursors in the hilus, $GABA_A$ receptor activation can lead to a depolarization of the membrane if the reversal potential for Cl^- is more depolarized than the resting potential, and this can gate other voltage dependent ion channels, such as Ca^{2+} channels. Interestingly, depolarizing $GABA_A$ receptor-mediated responses have been linked to developmental changes in neurons (LoTurco *et al*., 1995), and the $GABA_A$ agonist muscimol had affects similar to glutamate on the proliferation of NG2 cells in slice cultures (Yuan *et al*., 1998), suggesting that $GABA_A$ receptors also could be involved in oligodendrocyte development. These effects of "fast" neurotransmitters contrast with the effect of isoproterenol, a β adrenergic receptor agonist, which inhibited proliferation of O-2A cells, but stimulated lineage progression (Ghiani *et al*., 1999). These results raise the possibility that neurotransmitter receptors on NG2 cells may play an important role in regulating proliferation and maturation of NG2 cells in the mammalian CNS.

EXCITOXICITY AND DEMYELINATION

Although the expression of ionotropic glutamate receptors provides a pathway for rapid communication between neurons and NG2 cells, it also imparts a potential vulnerability to glutamate-induced excitotoxicity. The

inability of cells to cope with a continued influx of Ca^{2+} through Ca^{2+} permeable glutamate receptors and/or though voltage-gated Ca^{2+} channels during periods of sustained glutamate release can lead to excitotoxic damage and eventual degeneration. The expression of Ca^{2+} permeable glutamate receptors by NG2 cells suggests that they too could be damaged by prolonged activation of these receptors. Consistent with this hypothesis, exposure of O-2A cells or oligodendrocytes *in vitro* to glutamate, AMPA, or kainate caused extensive cell death that was prevented by NBQX (Matute *et al*., 2001; McDonald *et al*., 1998). NG2 cells may be susceptible to this form of excitotoxicity *in vivo*. Hypoxic/ischemic episodes are especially common in infants that are born prematurely, which must survive without a fully formed cerebral vasculature. Perinatal ischemia is most often associated with lesions in the white matter surrounding the ventricles, termed periventricular leukomalacia, that are characterized by widespread oligodendrocyte cell death and a resulting hypomyelination of axons (Back *et al*., 2001). This damage to axon tracts is the major pathology associated with cerebral palsy, the most common birth disorder. It is this inability of axons to transmit signals appropriately that is responsible for the profound motor deficits that characterize cerebral palsy (Volpe, 1998).

Because perinatal damage to white matter occurs when oligodendrocytes are developing from progenitors, much of the damage is likely to be sustained by immature oligodendrocytes and perhaps also NG2 cells (Back *et al*., 2001). Work in animal models suggests that there is a prolonged increase in extracellular glutamate levels during these ischemic episodes. These results suggest that a direct excitotoxic mechanism may contribute to the damage observed. The involvement of glutamate receptors in this damage to myelin is supported by the ability of kainate, an AMPA/kainate receptor agonist, to induce similar damage, and the ability of NBQX, a glutamate receptor antagonist, to inhibit both kainate-induced and ischemia-induced damage to white matter (Matute *et al*., 2001). These results raise the exciting possibility that AMPA/kainate receptor antagonists selective for NG2 cell glutamate receptors may be effective in preventing the damage to white matter that is associated with cerebral palsy. Experimentally induced white matter lesions in rat pups by combined carotid ligation and hypoxia or by infusion of AMPA were significantly attenuated by systemic administration of NBQX (Follett *et al*., 2000), lending support for this approach.

Conclusions and future directions

The data described above and that presented elsewhere in this issue support the conclusion that glial cells which express the NG2 proteoglycan comprise a class of non-neuronal cells distinct from astrocytes, oligodendrocytes, or microglia. These cells are widely distributed throughout the CNS, have a small cell body with fine radiating processes that extend into the neuropil. Among their defining physiological characteristics are a moderate input resistance, lack of gap junctional coupling, expression of numerous voltage-dependent ion channels and receptors for various neurotransmitters, and the formation of synaptic junctions with neurons. These cells share many properties with O-2A cells and complex cells, which have been subjected to more extensive physiological studies. Activation of neurotransmitter receptors on NG2 cells can inhibit K^+ channels and lead to changes in their proliferation and maturation, yet the presence of ionotropic glutamate receptors puts these cells at risk for excitotoxic damage during ischemia. Selective inhibition of these receptors could help limit white matter damage in premature infants and adults; however, as these receptors are similar to those expressed by neurons, considerable challenges exist for selective targeting of these NG2 cell glutamate receptors.

Many questions remain to be answered about the identity of this group of glial cells and their roles in brain physiology. Are these cells primarily involved in generating oligodendrocytes and forming myelin, or do they merely share a common lineage with true oligodendrocytes progenitors? Could these cells represent a form of multipotential precursor that is capable of differentiating into astrocytes, oligodendrocytes, or perhaps even neurons, depending on the types of signals being received, as suggested by recent results (Kondo & Raff, 2000)? Or, do these cells represent a specialized or immature form of astrocyte? As yet we do not have answers to these compelling questions. Future physiological studies will help to determine whether the properties of NG2-expressing glial cells are conserved in different brain regions, what role synaptic signaling plays in regulating their physiology and development, and whether NG2 cells are capable of transmitting signals back to neurons to influence neuronal activity.

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References

AKOPIAN, G., KRESSIN, K., DEROUICHE, A. & STEINHAUSER, C. (1996) Identified glial cells in the

early postnatal mouse hippocampus display different types of Ca2+ currents. *Glia* **17**, 181–194.

AKOPIAN, G., KUPRIJANOVA, E., KRESSIN, K. & STEINHAUSER, C. (1997) Analysis of ion channel expression by astrocytes in red nucleus brain stem slices. *Glia* **19**, 234–246.

ASZTELY, F., ERDEMLI, G. & KULLMANN, D. M. (1997) Extrasynaptic glutamate spillover in the hippocampus: Dependence on temperature and the role of active glutamate uptake. *Neuron* **18**, 281–293.

BACK, S., LUO, N. L., BORENSTEIN, N. S., LEVINE, J. M., VOLPE, J. J. & KINNEY, H. C. (2001) Late oligodendrocyte progenitors coincide with the developmental window of vulnerability for human perinatal white matter injury. *J. Neurosci*. **21**, 1302–1312.

BACKUS, K. H. & BERGER, T. (1995) Developmental variation of the permeability to Ca2+ of AMPA receptors in presumed hilar glial precursor cells. *Pflugers Arch*. **431**, 244–252.

BACKUS, K. H., ELSEN, F. P. & SCHULZE, C. H. (1998) Effect of kainite on the membrane conductance of hilar glial precursor cells recorded in the perforated-patch configuration. *Glia* **23**, 35–44.

BARRES, B. A., KOROSHETZ, W. J., CHUN, L. L. Y. & COREY, D. P. (1990a) Ion channel expression by white matter glia: The type-1 astrocyte. *Neuron* **5**, 527–544.

BARRES, B. A., KOROSHETZ, W. J., SWARTZ, K. J., CHUN, L. L. Y. & COREY, D. P. (1990b) Ion channel expression by white matter glia: The O-2A glial progenitor cell. *Neuron* **4**, 507–524.

BELACHEW, S., YUAN, X. & GALLO, V. (2001) Unraveling oligodendrocyte origin and function by cell-specific transgenesis. *Dev. Neurosci*. **23**, 287–298.

BERGER, T. (1995) AMPA-type glutamate receptors in glial precursor cells of the rat corpus callosum: Ionic and pharmacological properties. *Glia* **14**, 101–114.

BERGLES, D. E., ROBERTS, J. D. B., SOMOGYI, P. & JAHR, C. E. (2000) Glutamatergic synapses on oligodendrocyte precursor cells in the hippocampus. *Nature* **405**, 187–191.

BERGLES, D. E., DZUBAY, J. A. & JAHR, C. E. (1997) Glutamate transporter currents in bergmann glial cells follow the time course of extrasynaptic glutamate. *Proc Natl Acad Sci USA* **94**, 14821–5.23.

BERGLES, D. E. & JAHR, C. E. (1997) Synaptic activation of glutamate transporters in hippocampal astrocytes. *Neuron* **19**, 1297–1308.

BORDEY, A. & SONTHEIMER, H. (1997) Postnatal development of ionic currents in rat hippocampal astrocytes *in situ*. *J Neurophysiol*. **78**, 461–477.

BORGES, K. & KETTENMANN, H. (1995) Blockade of K+ channels induced by AMPA/kainate receptor activation in mouse oligodendrocyte precursor cells is mediated by Na+ entry. *J. Neurosci. Res*. **42**, 579–593

BOUCSEIN, C., KETTENMANN, H. & NOLTE, C. (2000) Electrophysiological properties of microglial cells in normal and pathologic rat brain slices. *Eur. J. Neurosci.* **12**, 2049–2058.

CHEN, Z. J., UGHRIN, Y. & LEVINE, J. M. (2002) Inhibition of axon growth by oligodendrocyte precursor cells. *Mol. Cell. Neurosci*. **20**, 125–139.

CLARK, B. A. & BARBOUR, B. (1997) Currents evoked in Bergmann glial cells by parallel fibre stimulation in rat cerebellar slices. *J. Physiol*. **502**, 335–350.

CLEMENTS, J. D., LESTER, R. A., TONG, G., JAHR, C. E. & WESTBROOK, G. L. (1992) The time course of glutamate in the synaptic cleft. *Science* **258**, 1498–1501.

D'AMBROSIO, R., WENZEL, J., SCHWARTZKROIN, P., McKHANN II, G. M. & JANIGRO, D. (1998) Functional specialization and topographic segregation of hippocampal astrocytes. *J. Neurosci*. **18**, 4425–4438.

DIERS-FENGER, M., KIRCHHOFF, F., KETTENMANN, H., LEVINE, J. M. & TROTTER, J. (2001) AN2/NG2 protein-expressing glial progenitor cells in the murine CNS: Isolation, differentiation, and association with radial glia. *Glia* **34**, 213–228.

DINGLEDINE, R., BORGES, K., BOWIE, D. & TRAYNELIS, S. F. (1999). The glutamate receptor ion channels. *Pharmacol. Rev.* **51**, 7–61.

DZUBAY, J. A. & JAHR, C. E. (1999) The concentration of synaptically released glutamate outside of the climbing fiber-Purkinje cell synaptic cleft. *J. Neurosci*. **19**, 5265–5274.

FOLLETT, P. L., ROSENBERG, P. A., VOLPE, J. J. & JENSEN, F. E. (2000) NBQX attenuates excitotoxic injury in developing white matter. *J. Neurosci*. **20**, 9235–9241.

FULTON, B. P., BURNE, J. F. & RAFF, M. C. (1992) Visualization of O-2A progenitor cells in developing and adult rat optic nerve by quisqualate-stimulated cobalt uptake. *J. Neurosci.* **12**, 4816–4833.

GALLO, V., ZHOU, J. M., McBAIN C. J., WRIGHT, P., KNUTSON, P. L. & ARMSTRONG, R. C. (1996) Oligodendrocyte progenitor cell proliferation and lineage progression are regulated by glutamate receptor-mediated K+ channel block. *J. Neurosci*. **16**, 2659–2670.

GHIANI, C. A., YUAN, X., EISEN, A. M., KNUTSON, P. L., DEPINHO, R. A., McBAIN, C. J. & GALLO, V. (1999) Voltage-activated K+ channels and membrane depolarization regulate accumulation of the cyclin-dependent kinase inhibitors p27(Kip1) and p21(CIP1) in glial progenitor cells. *J. Neurosci*. **19**, 5380–5392.

GIBSON, J. R., BEIERLEIN, M. & CONNORS, B. W. (1999) Two networks of electrically coupled inhibitory neurons in neocortex. *Nature* **402**, 75–79.

HOLZWARTH, J. A., GIBBONS, S. J., BRORSON, J. R., PHILIPSON, L. H. & MILLER, R. J. (1994) Glutamate receptor agonists stimulate diverse calcium responses in different types of cultured rat cortical glial cells. *J. Neurosci*. **14**, 1879–1891.

KANG, J., JIANG, L., GOLDMAN, S. A. & NEDERGAARD, M. (1998) Astrocyte-mediated potentiation of inhibitory synaptic transmission. *Nat. Neurosci* **1**, 683–692.

KONDO, T. & RAFF, M. (2000) Oligodendrocyte precursor cells reprogrammed to become multipotential CNS stem cells. *Science* **289**, 1754–1757.

KNUTSON, P., GHIANI, C. A., ZHOU, J. M., GALLO, V., & McBAIN, C. J. (1997) K+ channel expression and cell proliferation are regulated by intracellular sodium and membrane depolarization in oligodendrocyte progenitor cells. *J. Neurosci*. **17**, 2669–2682.

KRESSIN, K., KUPRIJANOVA, E., JABS, R., SEIFERT, G. & STEINHAUSER, C. (1995) Developmental regulation

of Na+ and K+ conductances in glial cells of mouse hippocampal slices. *Glia* **15**, 173–187.

LEVINE, J. M. & CHARD, J. P. (1987) Light and electron microscopic localization of a cell surface antigen (NG2) in the rat cerebellum: Association with smooth protoplasmic astrocytes. *J. Neurosci.* **7**, 2711–2720.

LEVINE, J. M. & NISHIYAMA, A. (1996) The NG2 chondroitin sulfate proteoglycan: A multifunctional proteoglycan associated with immature cells. *Perspect. Dev. Neurobiol*. **3**, 245–259.

LEVINE, J. M., REYNOLDS, R. & FAWCETT, J. W. (2001). The oligodendrocyte precursor cell in health and disease. *Trends in Neurosci.* **24**, 39–47.

LIU, H. N. & ALMAZAN, G. (1995) Glutamate induces c-fos proto-oncogene expression and inhibits proliferation in oligodendrocyte progenitors. *Eur. J. Neurosci*. **7**, 2355–2363.

LOTURCO, J. J., OWENS, D. F., HEATH, M. J. S., DAVIS, M. B. E. & KRIEGSTEIN, A. R. (1995) GABA and glutamate depolarize cortical progenitor cells and inhibit DNA synthesis. *Neuron* **15**, 1287–1298.

MALLON, B. S., SHICK, H. E., KIDD, G. J. & MACKLIN, W. B. (2002) Proteolipid promoter activity distinguishes two populations of NG2-positive cells throughout neonatal cortical development. *J. Neurosci*. **22**, 876–885.

MARRIOTT, D. R. & WILKIN, G. P. (1993) Substance P receptors on O-2A progenitor cells and type-2 astrocytes *in vitro*. *J. Neurochem*. **61**, 826–834.

MATUTE, C., ELBERDI, E., DOMERCQ, M., PEREZ-CERDA, F., PEREZ-SAMARTIN, A. & SANCHEZ-GOMEZ, M. V. (2001) The link between excitotoxic oligodendroglial death and demyelinating diseases. *Trends in Neurosci.* **24**, 224–230.

McDONALD, J. W., LEVINE, J. M. & QU, Y. (1998) Multiple classes of the oligodendrocyte lineage are highly vulnerable to excitotoxicity. *Neuroreport* **9**, 2757–2762.

NEUSCH, C., ROZENGURT, N., JACOBS, R. E., LESTER, H. A. & KOFUJI, P. (2001) Kir4.1 potassium channel subunit is crucial for oligodendrocyte development and *in vivo* myelination. *J. Neurosci*. **21**, 5429–5438.

NISHIYAMA, A., CHANG, A. & TRAPP, B. D. (1999) NG2+ glial cells: A novel glial cell population in the adult brain. *Neuropath. Exp. Neurol.* **58**, 1113–1124.

NISHIYAMA, A., YU, M., DRAZBA, J. A. & TUOHY, V. K. (1997) Normal and reactive NG2+ glial cells are distinct from resting and activated microglia. *J. Neurosci. Res*. **48**, 299–312.

NOLTE, C., MATYASH, M., PIVNEVA, T., SCHIPKE, C. G., OHLEMEYER, C., HANISCH, U. K., KIRCHHOFF, F. & KETTENMANN, H. (2001) GFAP promoter-controlled EGFP-expressing transgenic mice: A tool to visualize astrocytes and astrogliosis in living brain tissue. *Glia* **33**, 72–86.

OGATA, K. & KOSAKA, T. (2002) Structural and quantitative analysis of astrocytes in the mouse hippocampus. *Neuroscience* **113**, 221–233.

ONG, W. Y. & GAREY, L. J. (1996) A light and electron microscopic study of GluR4-positive cells in the human cerebral cortex. *Neurosci. Lett*. **210**, 107–110.

PARPURA, V. & HAYDON, P. G. (2000) Physiological astrocytic calcium levels stimulate glutamate release to modulate adjacent neurons. *Proc. Natl. Acad. Sci. USA* **97**, 8629–8634.

PARRI, H. R., GOULD, T. M. & CRUNELLI, V. (2001) Spontaneous astrocytic Ca2+ oscillations *in situ* drive NMDAR-mediated neuronal excitation. *Nat. Neurosci*. **4**, 803–812.

PATNEAU, D. K., WRIGHT, P. W., WINTERS, C., MAYER, M. L. & GALLO, V. (1994) Glial cells of the oligodendrocyte lineage express both kainate- and AMPA-preferring subtypes of glutamate receptor. *Neuron* **12**, 357–371.

POOPALASUNDARAM, S., KNOTT, C., SHAMOTIENKO, O. G., FORAN, P. G., DOLLY, J. O., GHIANI, C. A., GALLO, V. & WILKIN, G. P. (2000) Glial heterogeneity in expression of the inwardly rectifying K(+) channel, Kir4.1, in adult rat CNS. *Glia* **30**, 362–372.

PORTER, J. T. & McCARTHY, K. D. (1996) Hippocampal astrocytes *in situ* respond to glutamate released from synaptic terminals. *J. Neurosci*. **16**, 5073–5081.

RAFF, M. C., MILLER, R. H. & NOBLE, M. (1984) A glial progenitor cell that develops *in vitro* into an astrocyte or an oligodendrocyte depending on culture medium. *Nature* **303**, 390–396.

REIMANN, F. & ASHCROFT, F. M. (1999) Inwardly rectifying potassium channels. *Curr. Opin. Cell Biol*. **11**, 503–508.

ROGERS, S. W., GREGORI, N. Z., CARLSON, N., GAHRING, L. C. & NOBLE, M. (2001) Neuronal nicotinic acetylcholine receptor expression by O2A/oligodendrocyte progenitor cells. *Glia* **33**, 306–313.

SCHMIDT, K., EULITZ, D., VEH, R., KETTENMANN, H. & KIRCHHOFF, F. (1999) Heterogeneous expression of voltage-gated potassium channels of the shaker family (Kv1) in oligodendrocyte progenitors. *Brain Res*. **843**, 145–160.

SCHRODER, W., SEIFERT, G., HUTTMANN, K., HINTERKEUSER, S. & STEINHAUSER, C. (2002) AMPA receptor-mediated modulation of inward rectifier K+ channels in astrocytes of mouse hippocampus. *Mol. Cell Neurosci*. **19**, 447–458.

SEIFERT, G., LIBERTAD, R., WEBER, M. & STEINHAUSER, C. (1997a) AMPA receptor subunits expressed by single astrocytes in the juvenile mouse hippocampus. *Mol. Brain Res.* **47**, 286–294.

SEIFERT, G., ZHOU, M. & STEINHAUSER, C. (1997b) Analysis of AMPA recptor properties during postnatal development of mouse hippocampal astrocytes. *J. Neurophysiol*. **78**, 2916–2923.

STEINHAUSER, C., BERGER, T., FROTSCHER, M. & KETTENMANN, H. (1992) Heterogeneity in the membrane current pattern of identified glial cells in the hippocampal slice. *Eur. J. Neurosci.* **4**, 472–484.

STEINHAUSER, C., JABS, R. & KETTENMANN, H. (1994) Properties of GABA and glutamate responses in identified glial cells of the mouse hippocampal slice. *Hippocampus* **1**, 19–36.

TSE, F. W., FRASER, D. D., DUFFY, S. & MACVICAR, B. A. (1992) Voltage-gated K+ currents in acutely isolated hippocampal astrocytes. *J. Neurosci*. **12**, 1781–1788.

VOLPE, J. J. (1998) Brain injury in the premature infant: Overview of clinical aspects, neuropathology, and pathogenesis. *Semin. Pediatr. Neurol*. **5**, 135–151.

VON BLANKENFELD, G., TROTTER, J. & KETTENMANN, H. (1991) Expression and developmental regulation of a GABAa receptor in cultured murine cells of the oligodendrocyte lineage. *Eur. J. Neurosci*. **3**, 310–316.

WILLIAMSON, A. V., MELLOR, J. R., GRANT, A. L. & RANDALL, A. D. (1998) Properties of GABA(A) receptors in cultured rat oligodendrocyte progenitor cells. *Neuropharm*. **37**, 859–873.

YUAN, X., EISEN, A. X., McBAIN, C. J. & GALLO, V. (1998) A role for glutamate and its receptors in the regulation of oligodendrocyte development in cerebellar tissue slices. *Development* **125**, 2901–2914.

ZAWAR, C., PLANT, T. D., SCHIRRA, C., KONNERTH, A. & NEUMCKE, B. (1999) Cell-type specific expression of ATP-sensitive potassium channels in the rat hippocampus. *J. Physiol*. **514**, 327–341.

ZHOU, M. & KIMELBERG, H. K. (2001) Freshly isolated hippocampal CA1 astrocytes comprise two populations differing in glutamate transporter and AMPA receptor expression. *J. Neurosci*. **21**, 7901–7908.

ZHOU, M., SCHOOLS & KIMELBERG, H. K. (2000) GFAP mRNA positive glia acutely isolated from rat hippocampus predominantly show complex current patterns. *Brain Res. Mol. Brain Res*. **76**, 121–131.