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Synaptic Signaling Between Neurons and Glia

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ABSTRACT Rapid signaling between vertebrate neurons occurs primarily at synapses, intercellular junctions where quantal release of neurotransmitter triggers rapid changes in membrane conductance through activation of ionotropic receptors. Glial cells express many of these same ionotropic receptors, yet little is known about how receptors in glial cells become activated in situ. Because synapses were thought to be the sole provenance of neurons, it has been assumed that these receptors must be activated following diffusion of transmitter out of the synaptic cleft, or through nonsynaptic mechanisms such as transporter reversal. Two recent reports show that a ubiquitous class of progenitors that express the proteoglycan NG2 (NG2 cells) engage in rapid signaling with glutamatergic and γ-aminobutyric acid (GABA)ergic neurons through direct neuron-glia synapses. Quantal release of transmitter from neurons at these sites triggers rapid activation of aminomethylisoxazole propionic acid (AMPA) or $GABA_A$ receptors in NG2 cells. These currents exhibit properties consistent with direct rather than spillover-mediated transmission, and electron micrographic analyses indicate that nerve terminals containing clusters of synaptic vesicles form discrete junctions with NG2 cell processes. Although activation of AMPA or $GABA_A$ receptors depolarize NG2 cells, these receptors are more likely to serve as routes for ion flux rather than as current sources for depolarization, because the amplitudes of the synaptic transients are small and the resting membrane potential of NG2 cells is highly negative. The ability of both glutamate and GABA to influence the morphology, physiology, and development of NG2 cells in vitro suggests that this rapid form of signaling may play important roles in adapting the behavior of these cells to the needs of surrounding neurons in vivo.

INTRODUCTION

In the mammalian central nervous system, rapid communication between neurons is achieved mainly through synapses, specialized junctions where fast transmission of signals is ensured by close apposition, focal secretion, and clustering of receptors within the postsynaptic membrane. Activation of ionotropic receptors leads to rapid ion flux and membrane potential changes that are essential for invoking action potentials and triggering changes in gene expression, which can lead to alterations in morphology, plasticity, and excitability. Although glia are nonexcitable, they express many of the same receptors for neurotransmitters, including ionotropic receptors for glutamate and γ-aminobutyric acid (GABA) (Verkhratsky and Steinhauser, 2000), suggesting that conventional neurotransmitters may have broader roles in cell signaling. Application of receptor agonists to glial cells in vitro can induce membrane depolarizations, rises in the intracellular calcium, changes in proliferation, or even death, suggesting that the cellular physiology of glial

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cells may be subject to rapid regulation by neurons in situ (Hoppe and Kettenmann, 1989; Berger et al., 1992; Ziak et al., 1998; Alberdi et al., 2002). However, the mechanisms by which these receptors become activated in different classes of glia, and the responses that they invoke in vivo, remain poorly understood.

Because regulated fusion of transmitter-laden vesicles occurs at active zones, structures exclusive to synapses, it has been suggested that activation of receptors on glial cells must occur indirectly, when transmitter diffuses out of the synaptic cleft. This "spillout" of transmitter is the primary means of activation of metabotropic receptors in extra- or perisynaptic membranes (Baude et al., 1993), and transporters found in these regions are activated following transmitter release (Bergles et al., 1997; Bergles and Jahr, 1997; Otis et al., 1997; Kinney and Spain, 2002), suggesting that an indirect mechanism of activation is plausible. Astroglial cells (astrocytes and Bergmann glia) are particularly well positioned to respond to this spillout of transmitter as they are closely associated with synapses (Spacek, 1985; Ventura and Harris, 1999), and stimulation of excitatory afferents in the hippocampus and cerebellum induces activation of mGluRs (Porter and McCarthy, 1996), aminomethylisoxazole propionic acid (AMPA) receptors (Bergles et al., 1997; Clark and Barbour, 1997), and glutamate transporters (Bergles et al., 1997; Bergles and Jahr, 1997; Clark and Barbour, 1997) in these membranes. There is a growing appreciation that transmitters also can be released without vesicle fusion, through transporter activity (Kriegler and Chiu, 1993; Falkenburger et al., 2001; Baker et al., 2002) or by passing through certain receptor ion channels (Duan et al., 2003) or unpaired connexons (Ye et al., 2003), mechanisms aided by the large concentration gradient maintained for neurotransmitters. A confounding issue is the low affinity of AMPA and $GABA_A$ receptors. Transmitters are diluted rapidly in the extracellular space, placing extreme spatial and temporal constraints on the activation of these receptors. Because only one transmitter molecule is transported per cycle, and cycle times are typically >10 ms, transporter-dependent release is likely to produce only small changes in concentration under physiological conditions, inducing desensitization rather than activation of these ionotropic receptors (Trussell and Fischbach, 1989; Overstreet et al., 2000). Although channel-related mechanisms of release have the potential to produce more rapid and significant changes in concentration, whether these forms of release occur under physiological conditions in vivo remains unknown. Studies of NG2 cells, a class of progenitors widely distributed in the developing and adult brain, now indicate that ionotropic receptors are activated directly through the release of transmitter at neuron-glia synaptic junctions (Bergles et al., 2000; Lin and Bergles, 2004). This review describes electrophysiological and anatomical findings that support this conclusion and discusses the potential role of neuronal activity in regulating the behavior of these ubiquitous progenitors.

NEURON-NG2 CELL SYNAPTIC COMMUNICATION: CLASSIFICATION AND ELECTROPHYSIOLOGICAL PROPERTIES OF NG2 CELLS

Glial cells within the CNS are classified according to their physiological and anatomical features, and by their expression of different marker proteins. Immunohistochemical studies indicate that many small stellate-shaped cells in the CNS express NG2, a chondroitin sulfate proteoglycan, and the α-receptor for platelet-derived growth factor (PDGF-αR) (Levine and Card, 1987; Nishiyama et al., 1996). However, NG2 is also expressed by vasculature-associated cells, such as pericytes and smooth muscle cells (Ozerdem et al., 2001).

$NG2^+$/$PDGF^+$–αR cells in the CNS do not express traditional astrocyte-specific proteins, such glial fibrillary acidic protein (GFAP), microglia-specific proteins (e.g., OX-42), or proteins associated with mature oligodendrocytes (e.g., myelin basic protein [MBP]), indicating that they represent a distinct macroglial cell population (Nishiyama et al., 1999) (Fig. 1). These NG2 cells exhibit properties similar to oligodendrocyte-type-2 astrocyte (O-2A) cells, bipotential progenitors first identified in optic nerve (Raff et al., 1983) that differentiate into oligodendrocytes (Stallcup and Beasley, 1987) or "type 2" astrocytes in vitro, and have been classified as oligodendrocyte precursor cells (OPCs) (Levine et al., 2001). These cells express enhanced green fluorescent protein (EGFP) in mice engineered to express EGFP under control of the proteolipid protein (PLP) promoter (Mallon et al., 2002) or 2′-3′ cyclic nucleotide ′-phosphodiesterase (CNPase) promoter (Belachew et al., 2001), two proteins abundant in oligodendrocytes, and samples obtained from individual $NG2^+$ cells in the developing and adult cerebral cortex using laser capture microdissection contain mRNA for MBP and PLP, but not mRNAs for GFAP, neurofilament, or CD68. However, many NG2 cells in the hippocampus express S-100β in S-100β-EGFP reporter mice (Lin and Bergles, 2002; Vives et al., 2003), exhibit S-100β immunoreactivity (Matthias et al., 2003), and express EGFP weakly in GFAP-EGFP mice (Matthias et al., 2003). These results highlight the difficulty in classifying these cells, and may indicate that they comprise a heterogeneous group of progenitors with distinct developmental potential (see below).

NG2 cells exhibit physiological properties distinct from neurons and other classes of glial cells (Lin and Bergles, 2002). Although they rest close to the potassium equilibrium potential ($V_m \simeq -100$ mV), they have much higher membrane resistances (> 100 MΩ) than astrocytes (<10 MΩ) and they do not exhibit dye coupling through gap junctions (Bergles et al., 2000; Lin and Bergles, 2004). It is likely that many of the cells described in previous studies as "complex cells," "glial precursors," or "immature astrocytes," represent NG2 cells (Steinhauser et al., 1994; Akopian et al., 1996; Lin and Bergles, 2002; Matthias et al., 2003). Depolarizing voltage steps from the resting membrane potential

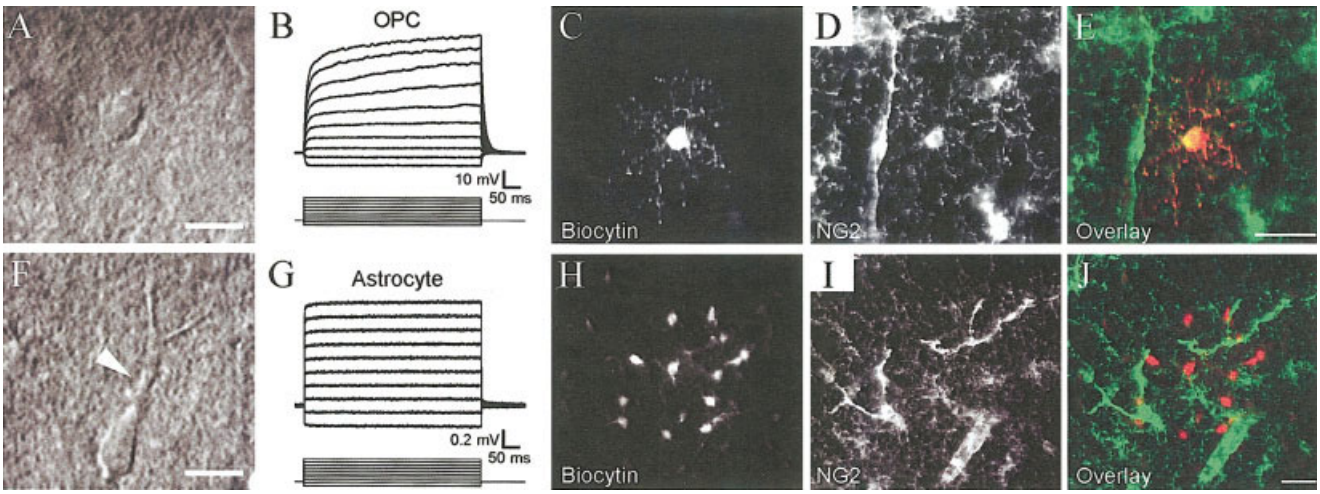

Fig. 1. NG2 cells are morphologically and physiologically distinct from astrocytes. **A:** Infrared-DIC image of an NG2 cell located in hippocampal CA1 stratum radiatum region (P14 rat). Note the lack of visible processes. **B:** Whole-cell current-clamp recording from a hippocampal NG2 cell. Injection of positive current did not induce action potentials (steps: −60–300 pA, 40-pA/step; KMeS-based electrode solution). **C:** Morphology of a representative NG2 cell filled with biocytin through the electrode. **D:** NG2 immunoreactivity of the same region of the slice shown in C. **E:** Overlay illustrating co-localization between biocytin and NG2, identifying this cell as an NG2 cell. **F:** Infrared-DIC image of an astrocyte located in hippocampal CA1 stratum radiatum (P14 rat). Arrowhead highlights a prominent process projecting from the soma. **G:** Current-clamp responses from a stratum radiatum astrocyte. **H:** Filling a single astrocyte with biocytin resulted in labeling of many nearby cells. **I:** NG2 immunoreactivity of the same region of the slice shown in H. **J:** Overlay illustrating that astrocytes do not exhibit NG2 immunoreactivity. Reprinted from Lin and Bergles (2004). Scale bars = 10 μm in A; 20 μm in C–E,H–J.

elicit A-type (I_A) and delayed-rectifier type (I_K) K^+ currents in NG2 cells, unlike the voltage-independent K^+ currents displayed by oligodendrocytes and astrocytes. Although astrocytes also express I_A and I_K, large leak K^+ currents and extensive gap-junction coupling typically obscure these currents. NG2 cells also express voltage-gated Na^+ channels that are similar in their kinetics and voltage dependencies to neuronal Na^+ channels, yet NG2 cells are nonexcitable. Apparently, the low density of these channels relative to K^+ channels prevents the initiation of regenerative spikes. It should be noted that some NG2 cells in the early postnatal brain (<P10) express fewer K^+ channels and exhibit regenerative activity, although these spikes are only elicited following considerable depolarization and have slower kinetics than action potentials in mature neurons (Chittajallu et al., 2003; J. Ziskin and D. Bergles, unpublished data). Thus, NG2 cells exhibit a unique combination of electrophysiological and anatomical features, which distinguish these cells from neurons and other macroglial cells in the CNS.

GLUTAMATERGIC SIGNALING WITH NG2 CELLS

In acute hippocampal slices prepared from young (P14–21) or adult rats (>P60), electrical stimulation of afferents in stratum radiatum of area CA1 elicits rapidly activating inward currents in NG2 cells of this region when they are voltage-clamped near their resting potential (Bergles et al., 2000). These currents begin with little delay after stimulation, suggesting that they are not mediated by metabotropic receptors. Indeed, these responses are blocked by the AMPA/kainate receptor antagonist NBQX and the selective AMPA receptor antagonist GYKI-53655, and are enhanced by cyclothiazide (Fig. 1A), indicating that they result from the activation of AMPA receptors. Furthermore, these evoked responses can be reversibly blocked by a low concentration of Cd^{2+} (30 μM) or tetrodotoxin (TTX), similar to excitatory postsynaptic currents (EPSCs) in neurons, indicating that action potential firing and Ca^{2+} influx are required to induce these responses; thus, they are unlikely to be produced by reverse transport of glutamate (Kriegler and Chiu, 1993; Zerangue and Kavanaugh, 1996). These EPSCs induce depolarizations that are about 2–10 mV in amplitude, raising questions about their physiological significance in light of the highly negative resting potential of NG2 cells. It appears that some of the AMPA receptors that underlie these currents are Ca^{2+}-permeable, as EPSCs in NG2 cells are inhibited by Joro spider toxin (Dingledine et al., 1999), and the current-to-voltage relationship of these responses exhibits inward rectification that is enhanced by inclusion of spermine in the recording electrode solution (Fig. 1B1 and 1B2) (Donevan and Rogawski, 1995). These ionotropic receptors may therefore serve primarily as a route for Ca^{2+} influx, rather than a source for depolarization.

How are AMPA receptors in NG2 cells activated following afferent stimulation? When recordings are made from NG2 cells in the presence of TTX, miniature EPSCs (mEPSCs) with rapid kinetics (<250 ms rise time, decay time <1 ms, at 22–24°C) are observed, indicating that spontaneous fusion of synaptic vesicles induces synchronous binding of glutamate to NG2 cell receptors. These responses are smaller in amplitude (average amplitude ≃−15 pA; V_m = −80 mV), occur

less frequently, and have more rapid kinetics than mEPSCs recorded from nearby CA1 pyramidal neurons. The rapid kinetics of these responses suggest that NG2 cell AMPA receptors are exposed to a high concentration of glutamate during the generation of mEPSCs, similar to that achieved in the synaptic cleft following fusion of a transmitter-loaded vesicle with the presynaptic membrane (Clements et al., 1992). These physiological data suggest that action potentials induce fusion of glutamate-filled vesicles directly opposite NG2 cell membranes where AMPA receptors are localized. This conclusion is supported by an electron microscopic analysis of physiologically identified NG2 cells. Using transmission electron microscopy, synaptic boutons containing clusters of vesicles were seen directly apposed to NG2 cell processes (Bergles et al., 2000). However, neuron-NG2 cell synaptic junctions differed from excitatory synapses on neurons: the presynaptic boutons were smaller, contained fewer vesicles, and the postsynaptic (NG2 cell) specialization was thinner and less well defined.

Where do these glutamatergic inputs originate? When two stimuli are applied with a short interval (~50 ms), evoked currents in NG2 cells exhibit facilitation similar to that displayed by Schaffer collateral-commissural axon-mediated EPSCs in CA1 pyramidal neurons. Furthermore, focal application of glutamate onto the cell body layer of area CA3 elicits brief bursts of EPSCs in NG2 cells located in the stratum radiatum of area CA1. These glutamate-evoked EPSCs are blocked by TTX, suggesting that they are dependent on propagation of action potentials from CA3 (Fig. 1C1 and 1C2). Because pyramidal neurons are the only glutamatergic neurons located in area CA3, these results suggest that the AMPA receptor EPSCs in NG2 cells arise from CA3 pyramidal neurons. These data are in accordance with EM studies indicating that some synaptic boutons in area CA1 formed junctions with both dendritic spines and NG2 cell processes (Bergles et al., 2000). Together, these electrophysiological and morphological data support the conclusion that the axons of CA3 pyramidal neurons form direct synaptic junctions with NG2 cells in the stratum radiatum region of area CA1.

Several recent studies suggest that transmitter exocytosis can also occur ectopically, at sites other than morphologically defined active zones (Zenisek et al., 2000; Lenzi et al., 2002; Matsui and Jahr, 2003). Could this phenomenon account for the quantal events recorded from NG2 cells? NG2 cell processes are often located perisynaptically, in direct contact with neuronal synapses (Ong and Levine, 1999; Bergles et al., 2000), and electron microscopic analyses indicate that some boutons that formed synapses with neuronal dendrites also were seen to form junctions with NG2 cell processes in subsequent sections (Bergles et al., 2000). However, ectopic release has only been shown to occur at specialized synapses that contain large numbers of vesicles and/or multiple active zones, such as climbing fiber synapses in the cerebellum, ribbon synapses in the cochlea and retina, and the calyx of held in the brainstem, while quantal release of transmitter onto NG2 cells has been resolved in many regions in which small, single active zone-containing synapses predominate (Lin et al., 2002). Furthermore, in the hippocampus, anatomically defined synaptic junctions were observed between neurons and NG2 cells that appeared to be exclusive, as these boutons did not form axospinous synapses in adjacent sections. These results do not exclude the possibility that NG2 cell ionotropic receptors are activated by ectopic release, but suggest that NG2 cells can be an exclusive target of axon collaterals.

The discovery of glutamatergic synaptic signaling in NG2 cells raises several new questions: (1) Are other neurotransmitter receptors on NG2 cells, such as $GABA_A$ receptors, activated by the same mechanism? (2) Is there any interaction among these different inputs? (3) Are neuron-NG2 cell synapses universal throughout the brain or unique to the hippocampus? (4) And, does this rapid signaling influence the physiology and perhaps the proliferation and development of NG2 cells?

GABAERGIC SIGNALING WITH NG2 CELLS

Previous studies have indicated that O-2A cells in vitro express $GABA_A$ receptors (Von Blankenfeld et al., 1991; Williamson et al., 1998), suggesting that NG2 cells also may communicate through GABAergic pathways. We found that focal application of THIP, a selective $GABA_A$ receptor agonist, to voltage-clamped NG2 cells in hippocampal slices elicited an inward current that was blocked by the $GABA_A$ receptor antagonist SR-95531 (gabazine), indicating that these cells express functional $GABA_A$ receptors in situ. To address how these receptors become activated physiologically, we electrically stimulated inhibitory afferents and recorded the resulting response in NG2 cells. When NG2 cells were loaded with Cl^- through the pipette solution to increase the size of the current flowing through $GABA_A$ receptor ion channels, we found that stimulation typically elicited a biphasic current in NG2 cells (Fig. 3A1). Much of the initial rapidly decaying current was blocked by NBQX, consistent with the activation of AMPA receptors as described above, while the slowly decaying current was blocked by the subsequent addition of gabazine (Fig. 3A2). Evoked $GABA_A$ receptor-mediated responses in NG2 cells also were dependent on both Ca^{2+} influx and action potential propagation, as they were blocked by TTX or Cd^{2+} (Fig. 3B). In addition, NG2 cell GABAergic responses showed paired-pulse depression (Fig. 3B), similar to evoked inhibitory postsynaptic currents in pyramidal neurons. In the absence of TTX, spontaneous $GABA_A$ receptor currents were observed in NG2 cells at a low frequency. The addition of carbachol, a muscarinic acetylcholine receptor agonist that potently depolarizes GABAergic interneurons and increases their firing rate (Pitler and Alger, 1992), significantly increased the frequency of spontaneous $GABA_A$ receptor currents in NG2 cells

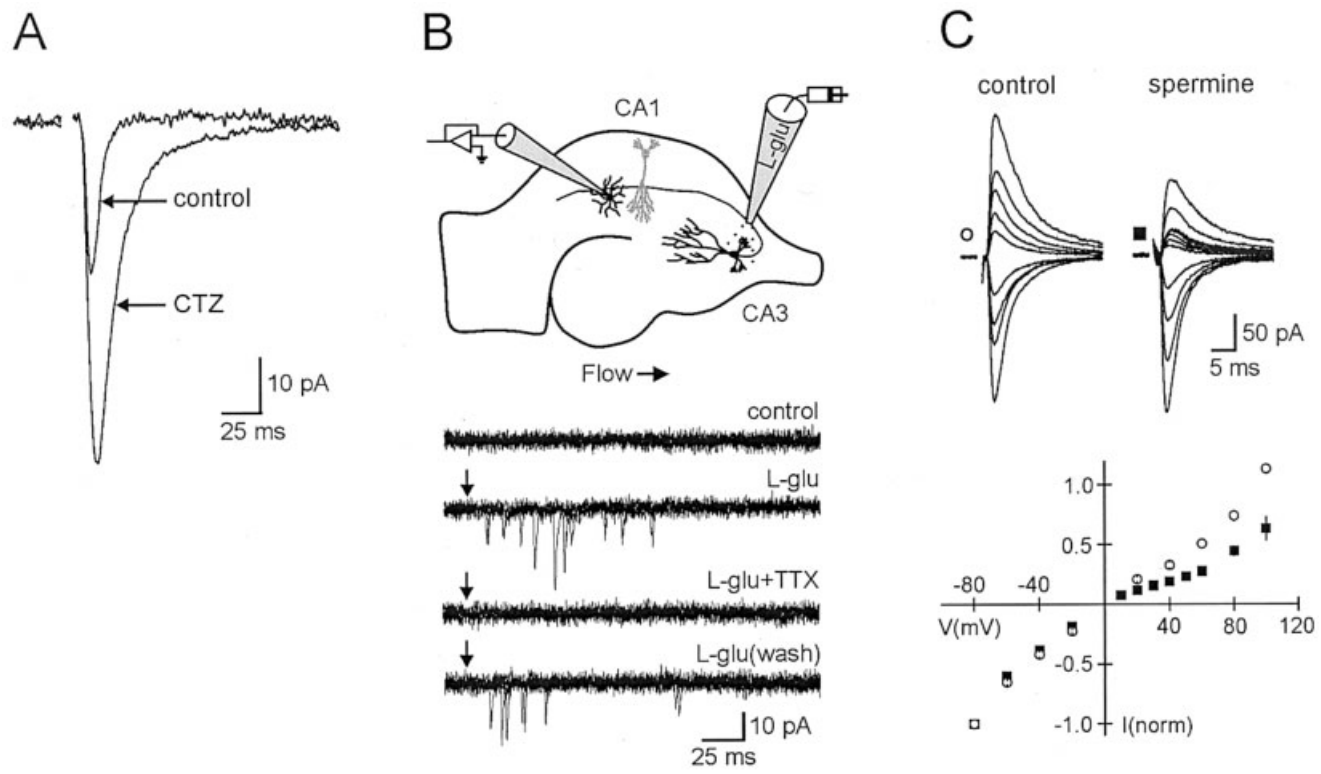

Fig. 2. Characteristics of rapid aminomethylisoxazole propionic acid (AMPA) receptor-mediated currents in hippocampal NG2 cells. **A:** Electrical stimulation in CA1 stratum radiatum elicited an inward current in an NG2 cell voltage-clamped at −80 mV. Bath application of cyclothiazide (CTZ, 200 μM) increased the amplitude and prolonged the decay of this evoked response. **B:** Top: Schematic illustration of recording configuration, with whole-cell recording of an NG2 cell in area CA1 and L-glutamate (200 μM) applied by pressure to area CA3. Bottom: Puffer application of L-glutamate (arrows) induced bursts of excitatory postsynaptic currents in an NG2 cell, that were reversibly blocked by tetrodotoxin (TTX, 1 μM). **C:** Top: Addition of spermine (25 μM) to the electrode solution decreased the amplitude of AMPA receptor-mediated currents at positive potentials. This polyamine-induced block caused the current-to-voltage relationship of evoked response to exhibit enhanced inward rectification (bottom). Reprinted with permission from Bergles et al. (2000).

(Fig. 3C1 and 3C2). This effect of carbachol was inhibited by TTX, indicating that the carbachol-induced enhancement reflected an increase in action potential-dependent events. The ability of carbachol to increase the frequency of these events suggests that the GABA reaching these receptors is released from local circuit interneurons within the hippocampal slice.

What is the mechanism for $GABA_A$ receptor activation in NG2 cells? In the presence of TTX, miniature $GABA_A$ receptor-mediated currents were visible in NG2 cells, demonstrating that these responses did not depend on the coincident activation of many adjacent terminals. Kinetic analysis of these currents revealed that they had an average rise time of about 0.8 ms, similar to miniature inhibitory postsynaptic currents (mIPSCs) recorded from CA1 pyramidal neurons, but decayed in about 24 ms (tau decay at 24°C), twice as slowly as pyramidal neuron mIPSCs (Fig. 3D1 and 3D2). NG2 cell miniature $GABA_A$ currents were also smaller (mean amplitude = −15 pA at −70 mV) and occurred much less frequently than mIPSCs in pyramidal neurons. NG2 cell GABA responses do not appear to result from spillover from nearby neuronal inhibitory synapses: They were not enhanced when GABA transporters were blocked, nor were they reduced in amplitude when GABA transporter activity was increased, as would be expected if they resulted from diffusion of GABA from nearby synapses. Agents that increase receptor affinity enhance spillover responses to a greater extent than responses mediated by direct release (Dzubay and Jahr, 1999), because the transmitter concentration experienced by receptors during spillover is much lower. We took advantage of the ability of benzodiazepines to increase the affinity of $GABA_A$ receptors to probe the occupancy of these receptors. Diazepam, a benzodiazepine that increases the affinity of $GABA_A$ receptors (Lavoie and Twyman, 1996), which has been used to assess the occupancy of $GABA_A$ receptors in neurons (Frerking et al., 1995), prolonged the decay of NG2 cell miniature $GABA_A$ currents but did not significantly change their amplitudes, effects similar to those observed on pyramidal neuron mIPSCs (Lin and Bergles, 2004). These data indicate that the occupancy of NG2 cell $GABA_A$ receptors is high following quantal release of GABA from interneuron terminals, a feat unlikely to be accomplished by spillover. These physiological data are supported by the co-localization of glutamic acid decarboxylase 65 (GAD65)-immunoreactive puncta and NG2 cell processes in this region of the hippocampus, and the occurrence in thin section electron micrographs of direct junctions between NG2 cell processes and nerve

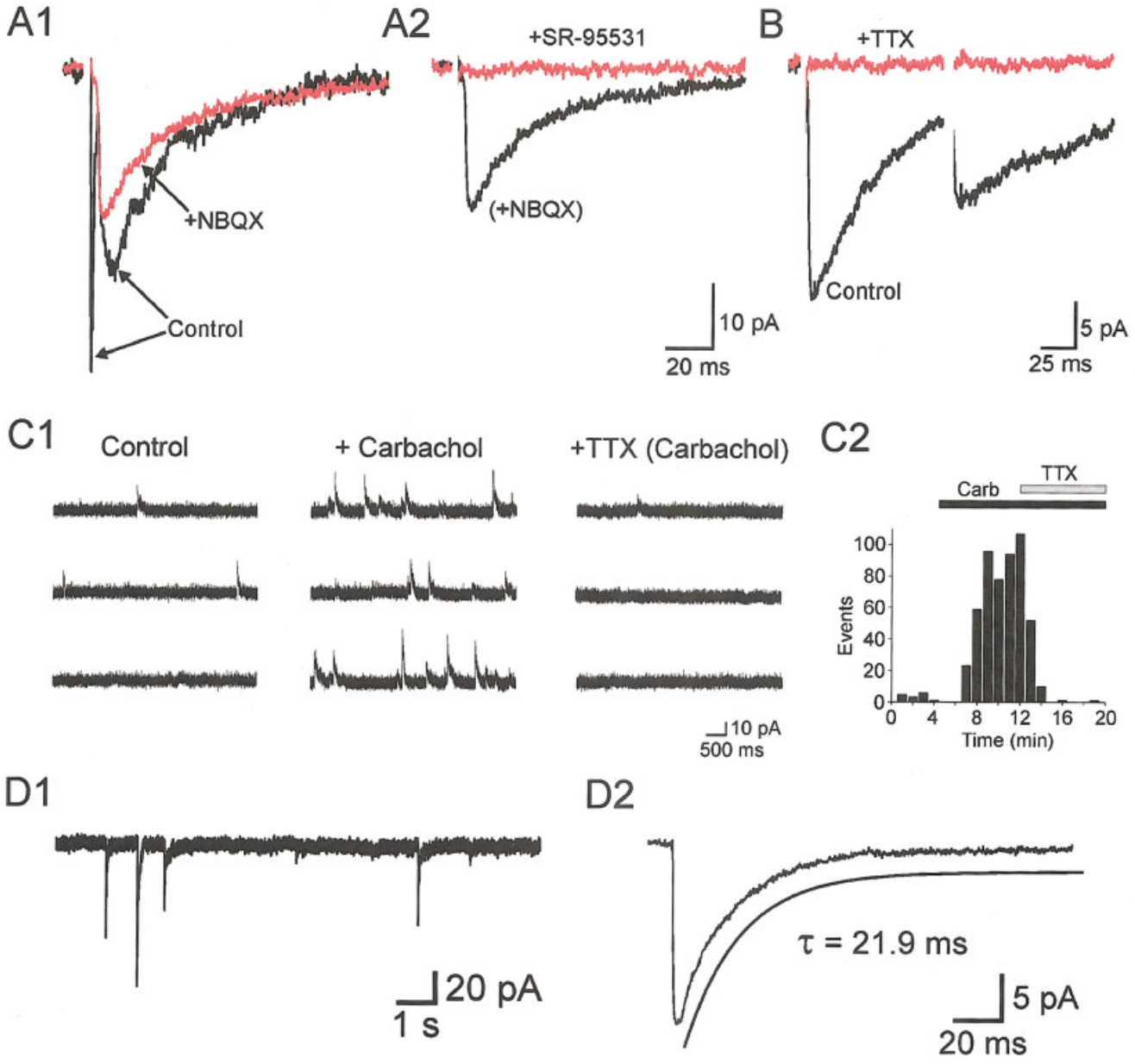

Fig. 3. Characteristics of γ-aminobutyric acid $(GABA)_A$ receptor-mediated currents in hippocampal NG2 cells. **A1:** Electrical stimulation in stratum radiatum of area CA1 elicited a biphasic inward current in an NG2 cell (CsCl-based electrode solution; $V_M = -70$ mV), which exhibited both rapidly decaying and slowly decaying components. The initial rapidly decaying component was blocked by NBQX (5 μM, red trace), indicating that it was mediated by aminomethylisoxazole propionic acid (AMPA) receptors. **A2:** The remaining slowly decaying component was blocked by SR-95531 (5 μM, red trace), indicating that it was mediated by $GABA_A$ receptors. Scales are the same in A1,2. **B:** Evoked $GABA_A$ receptor-mediated currents in NG2 cells exhibited paired-pulse depression (black trace), similar to monosynaptic IPSCs recorded from CA1 pyramidal neurons. The evoked response was also blocked by TTX (1 μM) (red trace), indicating that it was dependent on action potentials. The ACSF for this experiment contained 5 μM NBQX and 5 μM RS-CPP. **C1:** Increasing the firing rate of GABAergic interneurons by bath application of carbachol (5 μM) increased the frequency of spontaneous $GABA_A$ receptor-mediated currents in NG2 cells. For this experiment, the currents were outward because a low-chloride (cesium methanesulfonate-based; chloride concentration: 2 mM) electrode solution was used, and the cell was voltage-clamped at +30 mV. The carbachol-induced increase in spontaneous currents was blocked by TTX (1 μM). Each set of sweeps represents a continuous time period. **C2:** Plot of the frequency of spontaneous $GABA_A$ receptor-mediated currents over time for the experiment shown in C1. **D1:** Miniature $GABA_A$ receptor-mediated currents recorded from one NG2 cell (CsCl-based electrode solution; $V_M = -70$ mV) in the presence of TTX (1 μM). **D2:** Average time course of 64 miniature events recorded from the NG2 cell shown in D1. Reprinted from Lin and Bergles (2004).

terminals containing pleomorphic vesicles, characteristic of vesicles containing GABA.

Are these GABAergic inputs to NG2 cells inhibitory? Activation of $GABA_A$ receptors can depolarize or hyperpolarize a cell, depending on the resting potential and intracellular chloride concentration. During early development, neurons maintain a high intracellular chloride concentration, establishing a Cl^- reversal potential significantly more positive than the resting potential that allows GABA to exert an excitatory effect

(Owens et al., 1996; Dammerman et al., 2000). A previous study reported that application of GABA to cultured O-2A cells led to a rise of intracellular calcium, which was attributed to activation of voltage-gated Ca^{2+} channels by depolarizing $GABA_A$ receptor-mediated responses (Kirchhoff and Kettenmann, 1992). Using the gramicidin perforated-patch recording configuration, which does not disrupt $[Cl^-]_i$ (Kyrozis and Reichling, 1995), we found that $GABA_A$ receptor currents in hippocampal NG2 cells reversed at −43 mV, ~60 mV more depolarized than their resting potential (V_m = −102 mV). In contrast, $GABA_A$ receptor currents in pyramidal neurons reversed at −73 mV at this age (P14–21). These data suggest that GABA should have "excitatory" effects on NG2 cells. Indeed, application of a saturating dose of THIP depolarized NG2 cells by ~30 mV; however, this depolarization was not sufficient to evoke a detectable rise in $[Ca^{2+}]_i$ using Ca^{2+} imaging (D. Bergles and S. Lin, unpublished observations), and the largest $GABA_A$ receptor depolarizations mediated by spontaneous interneuron activity were ~5 mV. Although interneurons fire in high-frequency bursts in the developing hippocampus (Leinekugel et al., 2002), it seems unlikely that this activity would provide a depolarization sufficient to induce Ca^{2+} influx into NG2 cells. However, in younger NG2 cells, before the high resting conductance is established such activity may produce more profound depolarizations leading to activation of voltage-gated Ca^{2+} channels. $GABA_A$ receptors also may regulate AMPA receptor responses by shunting the current and/or by altering intracellular Cl^- (Van Damme et al., 2003). Consistent with this hypothesis, application of GABA to NG2 cells increased their membrane conductance and reduced the amplitude of AMPA receptor responses. Surprisingly, this inhibition persisted for several minutes after GABA was washed out and the resting conductance had returned to control levels, suggesting that $GABA_A$ receptor activation can induce a direct inhibition of AMPA receptors in NG2 cells.

NEURON-NG2 CELL SYNAPTIC SIGNALING IN OTHER BRAIN REGIONS

$NG2^+$ cells are found ubiquitously in both gray and white matter of the developing and adult CNS (Dawson et al., 2003). The discovery of rapid glutamatergic and GABAergic signaling in hippocampal NG2 cells led us to explore whether similar neuron-NG2 cell signaling occurs in other CNS regions. In order to identify NG2 cells outside the hippocampus, we used a transgenic mouse line in which the promoter of proteolipid protein (PLP) drives the expression of enhanced green fluorescent protein (EGFP) (hereafter referred as PLP-EGFP) (Fuss et al., 2000; Mallon et al., 2002). In PLP-EGFP mice, weakly $EGFP^+$ cells co-localize with NG2 in cortex, hippocampus, and cerebellum (Mallon et al., 2002); $EGFP^+$ cells that exhibit intense fluorescence are typically mature oligodendrocytes. Using EGFP as a guide, we have been able to identify and record from putative NG2 cells in the hippocampus, cortex, and cerebellum of the mouse (Lin et al., 2002), and confirmed that these cells were NG2 cells through retrospective immunohistochemistry against NG2. Electrical stimulation in these different regions elicited inward currents in NG2 cells that were blocked by NBQX and SR-95531 (when a Cl^- based internal solution was used). Furthermore, spontaneous and miniature currents mediated by AMPA and $GABA_A$ receptors were observed in NG2 cells from all regions. The rapid kinetics of these responses and their similarities to those observed in the rat hippocampus suggest that they arise from direct synaptic contacts between neurons and NG2 cells. Although we do not yet know the identity of the presynaptic neurons, or whether the properties of the receptors expressed by NG2 cells differs among these brain regions, these results suggest that rapid synaptic signaling is conserved among NG2 cells in the brain.

PHYSIOLOGICAL ROLES OF NEURON-NG2 CELL SYNAPTIC SIGNALING

The studies described above indicate that the resting potential of NG2 cells is essentially equal to E_K, more than 30 mV more hyperpolarized than neurons. This high resting potential, the small amplitudes of the synaptic responses, and the relatively high conductance of their membranes, decrease the likelihood that voltage-gated Ca^{2+} channels (Akopian et al., 1996; Kirchhoff and Kettenmann, 1992) would be activated in NG2 cells by the transient depolarizations resulting from synaptic activation of AMPA or $GABA_A$ receptors, particularly after the first postnatal week. Thus, these receptors are more likely to serve as a route for ion flux than as a current source for initiating gating of Ca^{2+} channels. It remains possible that the resting membrane conductance of NG2 cells and their membrane potential may be subject to modulation during different behavioral states, as they have been shown to express receptors for both acetylcholine (Nguyen et al., 2001) and norepinephrine (D. Perez, personal communication). Furthermore, prolonged exposure to GABA and glutamate has been shown to inhibit K^+ channels in glial cells (Borges et al., 1994; Jabs et al., 1994; Pastor et al., 1995; Schroder et al., 2002), which could amplify any transmitter-induced depolarization. A challenge of future studies will be to alter ionotropic receptor signaling selectively in NG2 cells to address how this form of neuron-glia signaling influences their physiology.

The ability to transduce activity between cells using neurotransmitters requires the appropriate expression and localization of many proteins, indicating that NG2 cells have made a significant investment in detecting the activity of surrounding neurons. Activation of AMPA or $GABA_A$ receptors on O-2A cells in vitro has been shown to induce Ca^{2+} influx, enhance expression of immediate early genes, and influence their proliferation and lineage progression (Kirchhoff and Kettenmann, 1992; Gallo et al., 1994, 1996; Knutson et al.,

1997), suggesting that these inputs may influence the development of these progenitors. NG2 cells constitute the principal dividing cell population in adult brain (Horner et al., 2000; Levine et al., 2001; Dawson et al., 2003), accounting for ~75% of all cortical cells that are pulse labeled with BrdU (Levine et al., 2001). Lineage studies that have used BrdU incorporation (Dawson et al., 2003) or clonal analysis following retroviral infection (Gensert and Goldman, 1996; Zhang and Goldman, 1996; Levison et al., 1999) to trace the fate of dividing cells in the mature brain indicate that these cells preferentially differentiate into oligodendrocytes in vivo, although they have a great capacity for self renewal. Recent studies suggest, however, that this may not be the only fate of NG2 cells, as progenitors (in some cases $NG2^+$) isolated from both gray and white matter have the ability to differentiate into neurons in vitro when exposed to fetal calf serum (FCS), PDGF, and basic fibroblast growth factor (bFGF) (Kondo and Raff, 2000; Shihabuddin et al., 2000; Nunes et al., 2003). Furthermore, inhibitory neurons express EGFP weakly in CNPase-EGFP mice, and some $NG2^+$ cells in the dentate gyrus express TOAD-64 (turned on after division, 64 kD), a protein expressed by early postmitotic neurons (Belachew et al., 2003). These results raise the possibility that some $NG2^+$ cells in the hippocampus may develop into GABAergic interneurons; there may be local factors that influence the fate of NG2 cells, or $NG2^+$ progenitors may consist of several different cell types (Mallon et al., 2002; Belachew et al., 2003) with distinct developmental potential (see reviews by Goldman, 2003; Horner et al., 2002). Our studies indicate that transmitter-dependent signaling between neurons and NG2 cells is not restricted to the developing brain, but is prominent in the adult CNS as well. An important question to address in future studies is whether this rapid signaling between neurons and NG2 cells influences their development or fate in vivo, or whether this signaling has other more direct roles in regulating the activity of surrounding neurons.

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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 Ca^{2+} currents. Glia 17:181–194.

Alberdi E, Sanchez-Gomez MV, Marino A, Matute C. 2002. Ca^{2+} influx through AMPA or kainate receptors alone is sufficient to initiate excitotoxicity in cultured oligodendrocytes. Neurobiol Dis 9:234–243.

Baker DA, Xi ZX, Shen H, Swanson CJ, Kalivas PW. 2002. The origin and neuronal function of in vivo nonsynaptic glutamate. J Neurosci 22:9134–9141.

Baude A, Nusser Z, Roberts JD, Mulvihill E, McIlhinney RA, Somogyi P. 1993. The metabotropic glutamate receptor (mGluR1 alpha) is concentrated at perisynaptic membrane of neuronal subpopulations as detected by immunogold reaction. Neuron 11:771–787.

Belachew S, Yuan X, Gallo V. 2001. Unraveling oligodendrocyte origin and function by cell-specific transgenesis. Dev Neurosci 23:287–298.

Belachew S, Chittajallu R, Aguirre AA, Yuan X, Kirby M, Anderson S, Gallo V. 2003. Postnatal NG2 proteoglycan-expressing progenitor cells are intrinsically multipotent and generate functional neurons. J Cell Biol 161:169–186.

Berger T, Walz W, Schnitzer J, Kettenmann H. 1992. GABA- and glutamate-activated currents in glial cells of the mouse corpus callosum slice. J Neurosci Res 31:21–27.

Bergles DE, Jahr CE. 1997. Synaptic activation of glutamate transporters in hippocampal astrocytes. Neuron 19:1297–308.

Bergles DE, Dzubay JA, Jahr CE. 1997. Glutamate transporter currents in Bergmann glial cells follow the time course of extrasynaptic glutamate. Proc Natl Acad Sci USA 94:14821–14825.

Bergles DE, Roberts JD, Somogyi P, Jahr CE. 2000. Glutamatergic synapses on oligodendrocyte precursor cells in the hippocampus. Nature 405:187–191.

Borges K, Ohlemeyer C, Trotter J, Kettenmann H. 1994. AMPA/kainate receptor activation in murine oligodendrocyte precursor cells leads to activation of a cation conductance, calcium influx and blockade of delayed rectifying K^+ channels. Neuroscience 63:135–149.

Chittajallu R, Aguirre AA, and Gallo V. 2003. Heterogeneous physiological properties of white and gray matter NG2 cells. Soc Neurosci Abs 455.1.

Clark BA, Barbour B. 1997. Currents evoked in Bergmann glial cells by parallel fibre stimulation in rat cerebellar slices. J Physiol (Lond) 502(Pt 2):335–350.

Clements JD, Lester RA, Tong G, Jahr CE, Westbrook GL. 1992. The time course of glutamate in the synaptic cleft. Science 258:1498–1501.

Dammerman RS, Flint AC, Noctor S, Kriegstein AR. 2000. An excitatory GABAergic plexus in developing neocortical layer 1. J Neurophysiol 84:428–434.

Dawson MR, Polito A, Levine JM, Reynolds R. 2003. NG2-expressing glial progenitor cells: an abundant and widespread population of cycling cells in the adult rat CNS. Mol Cell Neurosci 24:476–488.

Dingledine R, Borges K, Bowie D, Traynelis SF. 1999. The glutamate receptor ion channels. Pharmacol Rev 51:7–61.

Donevan SD, Rogawski MA. 1995. Intracellular polyamines mediate inward rectification of Ca^{2+}-permeable alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors. Proc Natl Acad Sci USA 92:9298–9302.

Duan S, Anderson CM, Keung EC, Chen Y, Swanson RA. 2003. P2X7 receptor-mediated release of excitatory amino acids from astrocytes. J Neurosci 23:1320–1328.

Dzubay JA, Jahr CE. 1999. The concentration of synaptically released glutamate outside of the climbing fiber-Purkinje cell synaptic cleft. J Neurosci 19:5265–5274.

Falkenburger BH, Barstow KL, Mintz IM. 2001. Dendrodendritic inhibition through reversal of dopamine transport. Science 293: 2465–2470.

Frerking M, Borges S, Wilson M. 1995. Variation in GABA mini amplitude is the consequence of variation in transmitter concentration. Neuron 15:885–895.

Fuss B, Mallon B, Phan T, Ohlemeyer C, Kirchhoff F, Nishiyama A, Macklin WB. 2000. Purification and analysis of in vivo-differentiated oligodendrocytes expressing the green fluorescent protein. Dev Biol 218:259–274.

Gallo V, Patneau DK, Mayer ML, Vaccarino FM. 1994. Excitatory amino acid receptors in glial progenitor cells: molecular and functional properties. Glia 11:94–101.

Gallo V, Zhou JM, McBain CJ, Wright P, Knutson PL, Armstrong RC. 1996. Oligodendrocyte progenitor cell proliferation and lineage progression are regulated by glutamate receptor-mediated K^+ channel block. J Neurosci 16:2659–2570.

Gensert JM, Goldman JE. 1996. In vivo characterization of endogenous proliferating cells in adult rat subcortical white matter. Glia 17:39–51.

Goldman S. 2003. Glia as neural progenitor cells. Trends Neurosci 26:590–596.

Hoppe D, Kettenmann H. 1989. GABA triggers a Cl^- efflux from cultured mouse oligodendrocytes. Neurosci Lett 97:334–339.

Horner PJ, Power AE, Kempermann G, Kuhn HG, Palmer TD, Winkler J, Thal LJ, Gage FH. 2000. Proliferation and differentiation of progenitor cells throughout the intact adult rat spinal cord. J Neurosci 20:2218–2228.

Horner PJ, Thallmair M, Gage FH. 2002. Defining the NG2-expressing cell of the adult CNS. J Neurocytol 31:469–480.

Jabs R, Kirchhoff F, Kettenmann H, Steinhauser C. 1994. Kainate activates Ca^{2+}-permeable glutamate receptors and blocks voltage-gated K^+ currents in glial cells of mouse hippocampal slices. Pflugers Arch 426:310–319.

Kinney GA, Spain WJ. 2002. Synaptically evoked GABA transporter currents in neocortical glia. J Neurophysiol 88:2899–2908.

Kirchhoff F, Kettenmann H. 1992. GABA triggers a $[Ca^{2+}]_i$ increase in murine precursor cells of the oligodendrocyte lineage. Eur J Neurosci 4:1049–1058.

Knutson P, Ghiani CA, Zhou JM, Gallo V, McBain CJ. 1997. K^+ channel expression and cell proliferation are regulated by intracellular sodium and membrane depolarization in oligodendrocyte progenitor cells. J Neurosci 17:2669–2682.

Kondo T, Raff M. 2000. Oligodendrocyte precursor cells reprogrammed to become multipotential CNS stem cells. Science 289: 1754–1757.

Kriegler S, Chiu SY. 1993. Calcium signaling of glial cells along mammalian axons. J Neurosci 13:4229–4245.

Kyrozis A, Reichling DB. 1995. Perforated-patch recording with gramicidin avoids artifactual changes in intracellular chloride concentration. J Neurosci Methods 57:27–35.

Lavoie AM, Twyman RE. 1996. Direct evidence for diazepam modulation of GABAA receptor microscopic affinity. Neuropharmacology 35:1383–1392.

Leinekugel X, Khazipov R, Cannon R, Hirase H, Ben-Ari Y, Buzsaki G. 2002. Correlated bursts of activity in the neonatal hippocampus in vivo. Science 296:2049–2052.

Lenzi D, Crum J, Ellisman MH, Roberts WM. 2002. Depolarization redistributes synaptic membrane and creates a gradient of vesicles on the synaptic body at a ribbon synapse. Neuron 36:649–659.

Levine JM, Card JP. 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 JM, Reynolds R, Fawcett JW. 2001. The oligodendrocyte precursor cell in health and disease. Trends Neurosci 24:39–47.

Levison SW, Young GM, Goldman JE. 1999. Cycling cells in the adult rat neocortex preferentially generate oligodendroglia. J Neurosci Res 57:435–446.

Lin SC, Bergles DE. 2002. Physiological characteristics of NG2-expressing glial cells. J Neurocytol 31:537–549.

Lin SC, Bergles DE. 2004. Synaptic signaling between GABAergic interneurons and oligodendrocyte precursor cells in the hippocampus. Nat Neurosci 7:24–32.

Lin S-C, Macklin W, and Bergles DE. 2002. Neurons throughout the brain communicate with oligodendrocyte precursor cells via direct synapses. Soc Neurosci Abs 747.1.

Mallon BS, Shick HE, Kidd GJ, Macklin WB. 2002. Proteolipid promoter activity distinguishes two populations of NG2-positive cells throughout neonatal cortical development. J Neurosci 22:876–885.

Matsui K, Jahr CE. 2003. Ectopic release of synaptic vesicles. Neuron 40:1173–1183.

Matthias K, Kirchhoff F, Seifert G, Huttmann K, Matyash M, Kettenmann H, Steinhauser C. 2003. Segregated expression of AMPA-type glutamate receptors and glutamate transporters defines distinct astrocyte populations in the mouse hippocampus. J Neurosci 23:1750–1758.

Nguyen L, Rigo JM, Rocher V, Belachew S, Malgrange B, Rogister B, Leprince P, Moonen G. 2001. Neurotransmitters as early signals for central nervous system development. Cell Tissue Res 305:187–202.

Nishiyama A, Lin XH, Giese N, Heldin CH, Stallcup WB. 1996. Co-localization of NG2 proteoglycan and PDGF alpha-receptor on O2A progenitor cells in the developing rat brain. J Neurosci Res 43:299–314.

Nishiyama A, Chang A, Trapp BD. 1999. $NG2^+$ glial cells: a novel glial cell population in the adult brain. J Neuropathol Exp Neurol 58: 1113–1124.

Nunes MC, Roy NS, Keyoung HM, Goodman RR, McKhann G II, Jiang L, Kang J, Nedergaard M, Goldman SA. 2003. Identification and isolation of multipotential neural progenitor cells from the subcortical white matter of the adult human brain. Nat Med 9:439–447.

Ong WY, Levine JM. 1999. A light and electron microscopic study of NG2 chondroitin sulfate proteoglycan-positive oligodendrocyte precursor cells in the normal and kainate-lesioned rat hippocampus. Neuroscience 92:83–95.

Otis TS, Kavanaugh MP, Jahr CE. 1997. Postsynaptic glutamate transport at the climbing fiber-Purkinje cell synapse. Science 277: 1515–1518.

Overstreet LS, Jones MV, Westbrook GL. 2000. Slow desensitization regulates the availability of synaptic $GABA_A$ receptors. J Neurosci 20:7914–7921.

Owens DF, Boyce LH, Davis MB, Kriegstein AR. 1996. Excitatory GABA responses in embryonic and neonatal cortical slices demonstrated by gramicidin perforated-patch recordings and calcium imaging. J Neurosci 16:6414–6423.

Ozerdem U, Grako KA, Dahlin-Huppe K, Monosov E, Stallcup WB. 2001. NG2 proteoglycan is expressed exclusively by mural cells during vascular morphogenesis. Dev Dyn 222:218–227.

Pastor A, Chvatal A, Sykova E, Kettenmann H. 1995. Glycine- and GABA-activated currents in identified glial cells of the developing rat spinal cord slice. Eur J Neurosci 7:1188–1198.

Pitler TA, Alger BE. 1992. Cholinergic excitation of GABAergic interneurons in the rat hippocampal slice. J Physiol 450:127–142.

Porter JT, McCarthy KD. 1996. Hippocampal astrocytes in situ respond to glutamate released from synaptic terminals. J Neurosci 16:5073–5081.

Raff MC, Miller RH, Noble M. 1983. A glial progenitor cell that develops in vitro into an astrocyte or an oligodendrocyte depending on culture medium. Nature 303:390–396.

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.

Shihabuddin LS, Horner PJ, Ray J, Gage FH. 2000. Adult spinal cord stem cells generate neurons after transplantation in the adult dentate gyrus. J Neurosci 20:8727–8735.

Spacek J. 1985. Three-dimensional analysis of dendritic spines. III. Glial sheath. Anat Embryol (Berl) 171:245–252.

Stallcup WB, Beasley L. 1987. Bipotential glial precursor cells of the optic nerve express the NG2 proteoglycan. J Neurosci 7:2737–2744.

Steinhauser C, Jabs R, Kettenmann H. 1994. Properties of GABA and glutamate responses in identified glial cells of the mouse hippocampal slice. Hippocampus 4:19–35.

Trussell LO, Fischbach GD. 1989. Glutamate receptor desensitization and its role in synaptic transmission. Neuron 3:209–218.

Van Damme P, Callewaert G, Eggermont J, Robberecht W, Van Den Bosch L. 2003. Chloride influx aggravates Ca^{2+}-dependent AMPA receptor-mediated motoneuron death. J Neurosci 23:4942–4950.

Ventura R, Harris KM. 1999. Three-dimensional relationships between hippocampal synapses and astrocytes. J Neurosci 19:6897–6906.

Verkhratsky A, Steinhauser C. 2000. Ion channels in glial cells. Brain Res Brain Res Rev 32:380–412.

Vives V, Alonso G, Solal AC, Joubert D, Legraverend C. 2003. Visualization of S100B-positive neurons and glia in the central nervous system of EGFP transgenic mice. J Comp Neurol 457:404–419.

Von Blankenfeld G, Trotter J, Kettenmann H. 1991. Expression and developmental regulation of a $GABA_A$ receptor in cultured murine cells of the oligodendrocyte lineage. Eur J Neurosci 3:310–316.

Williamson AV, Mellor JR, Grant AL, Randall AD. 1998. Properties of $GABA_A$ receptors in cultured rat oligodendrocyte progenitor cells. Neuropharmacology 37:859–873.

Ye ZC, Wyeth MS, Baltan-Tekkok S, Ransom BR. 2003. Functional hemichannels in astrocytes: a novel mechanism of glutamate release. J Neurosci 23:3588–3596.

Zenisek D, Steyer JA, Almers W. 2000. Transport, capture and exocytosis of single synaptic vesicles at active zones. Nature 406:849–854.

Zerangue N, Kavanaugh MP. 1996. Flux coupling in a neuronal glutamate transporter. Nature 383:634–637.

Zhang L, Goldman JE. 1996. Developmental fates and migratory pathways of dividing progenitors in the postnatal rat cerebellum. J Comp Neurol 370:536–550.

Ziak D, Chvatal A, Sykova E. 1998. Glutamate-, kainate- and NMDA-evoked membrane currents in identified glial cells in rat spinal cord slice. Physiol Res 47:365–375.