Synaptic communication between neurons and NG2$^+$ cells

Martin Paukert and Dwight E Bergles

Chemical synaptic transmission provides the basis for much of the rapid signaling that occurs within neuronal networks. However, recent studies have provided compelling evidence that synapses are not used exclusively for communication between neurons. Physiological and anatomical studies indicate that a distinct class of glia known as NG2$^+$ cells also forms direct synaptic junctions with both glutamatergic and GABAergic neurons. Glutamatergic signaling can influence intracellular Ca^{2+} levels in NG2$^+$ cells by activating Ca^{2+} permeable AMPA receptors, and these inputs can be potentiated through high frequency stimulation. Although the significance of this highly differentiated form of communication remains to be established, these neuro–glia synapses might enable neurons to influence rapidly the behavior of this ubiquitous class of glial progenitors.

Addresses
Department of Neuroscience, Johns Hopkins University School of Medicine, Baltimore, MD 21205, USA

Corresponding author: Bergles, Dwight E (dbergles@jhmi.edu)

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Introduction

A wealth of anatomical [1,2] and physiological [3] evidence supports the existence of a third class of macroglia (see glossary) in the CNS that have features distinct from astrocytes and oligodendrocytes. These stellate-shaped glial cells are uniquely identified by the expression of both the chondroitin sulfate proteoglycan NG2 and the α receptor for platelet-derived growth factor (PDGFαR). Immunolabeling for these antigens has revealed that these small glial cells are widely distributed in both white and gray matter of the mature nervous system, where they account for between 5 and 10% of all cells, depending on the brain region [4] (Figure 1). These glia are distinct from astrocytes, as they do not express glutamate transporters, glial fibrillary acidic protein (GFAP), or exhibit extensive coupling through gap junctions, and they are distinct from mature oligodendrocytes, as they do not express myelin basic protein (MBP) or form myelin sheaths. In previous reports these cells have been termed 'NG2 glia', 'NG2$^+$ cells', 'oligodendrocyte precursor cells (OPCs)', 'polydendrocytes', 'synantocytes', and 'complex cells', to distinguish them from other types of glial cells. In this review we use the term NG2$^+$ cells, although it remains possible that this general classification encompasses a greater number of distinct cell types.

NG2$^+$ cells function as progenitors for myelinating oligodendrocytes during development and following demyelination [5], and both *in vitro* and *in vivo* experiments suggest that they also have the capability to differentiate into astrocytes and neurons [6,7]. However, the persistence of these cells in both white and gray matter throughout life suggests that NG2$^+$ cells are not merely preserved as a pool of precursors from which to generate mature glia and neurons. Indeed, recent studies suggest that NG2$^+$ cells that reside in mature white matter might limit axon sprouting at nodes of Ranvier by delivering oligodendrocyte myelin glycoprotein (OMgp), a proteoglycan that influences neurite outgrowth [8•]. Nevertheless, many questions remain about the functions of these ubiquitous glial cells within the mature CNS.

Similar to astrocytes, NG2$^+$ cells express receptors for neurotransmitters [3,9], indicating that their behavior is likely to be influenced by neuronal activity. By understanding how they communicate with surrounding neurons — the patterns of activity required for activation of their receptors and the characteristics of the responses that are generated — we will be in a better position to understand the roles played by these enigmatic cells. Although indirect modes of activation, such as spillover or volume transmission (see glossary), were thought to be responsible for activation of receptors on glia [10], both glutamate and γ-amino butyric acid (GABA) receptors in NG2$^+$ cells are activated by vesicular release at defined chemical synapses [11,12,13••], which overturns the dogma that fast synaptic transmission occurs exclusively between neurons. In this review, we focus on new findings related to direct synaptic communication between neurons and NG2$^+$ cells, and conclude with a brief discussion of the differences between this mode of signaling and ectopic release of transmitter, which is responsible for activation of glutamate receptors in Bergmann glia [14].

Synapses are formed with NG2$^+$ cells in the hippocampus

NG2$^+$ cells throughout the brain extend a dozen or more thin processes 30–50 μm into the surrounding neuropil, which might enable them to interact with cells in this limited area. Within the hippocampus, NG2$^+$ cells are abundant in the strata where excitatory synapses are

Glossary

Ammon's horn sclerosis: A segmental loss of pyramidal neurons, granule cell dispersion and reactive gliosis seen in more than half of the epileptic brains examined at autopsy. It is still controversial whether hippocampal sclerosis is cause or consequence of seizure activity.

Astrogliosis: Astrocytes react to many pathologic stimuli with proliferation and hypertrophy, often leading to the formation of a glial scar.

EPSC rectification ratio: Any deviation from a linear current to voltage relation is designated as rectification. Intracellular polyamines can bind to and block AMPA receptors that are permeable to Ca^{2+} (lack GluR2 subunits). Because of their positive charges, polyamines bind more readily at depolarized membrane potentials, leading to a stronger reduction of outward currents. As a result, the smaller the outward current/inward current ratio at given potentials, the higher the proportion of Ca^{2+} permeable AMPA receptors.

Flip/Flop alternative splicing: The four AMPA receptor subunits (GluR1–4) exist in two splice variants, designated Flip and Flop, which vary by 9 – 11 amino acids, depending on the particular subunit. This alternative splicing leads to receptors that exhibit distinct pharmacological and kinetic properties.

Macroglia: Glial cells in the CNS include the bone marrow-derived microglia (''small glia'') and the neuroectoderm-derived macroglia (''large glia''). Macroglia is traditionally subdivided into two classes: astrocytes and myelin-forming oligodendrocytes.

Pleomorphic vesicles: Pleomorphic meaning 'in multiple shapes'. In contrast to glutamate-containing synaptic vesicles, which appear in electron microscopic images as round vesicles with an electron dense core, GABA-containing synaptic vesicles typically appear pleomorphic.

Shunting: A mode of inhibition whereby the opening of ion channels leads to a decrease in membrane resistance that limits membrane depolarization in response to another input.

Spillover: A phenomenon whereby neurotransmitter released at one synapse diffuses out of the cleft and activates receptors at a neighboring synapse.

Theta burst stimulation: Usually four to five stimuli applied at 100 Hz constitute one burst, and bursts are repeated every 200 ms. This pattern of stimulation is thought to mimic the activity experienced by fibers during theta oscillations *in vivo*.

Volume transmission: Transmitter release at sites where there is no classical postsynaptic structure. This mode of transmission has been proposed for the release of neuromodulators such as norepinephrine.

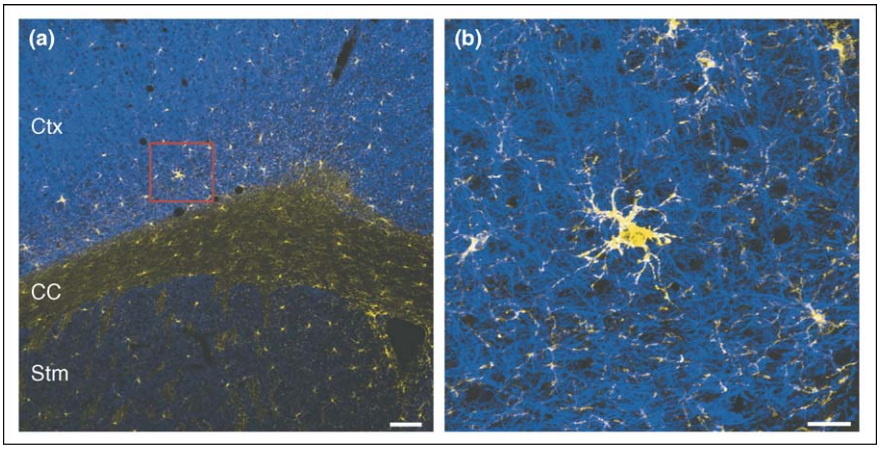
formed (radiatum, lacunosum moleculare, oriens). Whole cell patch clamp recordings from these cells in acute hippocampal slices indicate that similar to their *in vitro* correlate, the oligodendrocyte-type-2 astrocyte (O-2A) cells, they express AMPA (alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid) receptors [15,16]. A hallmark of synaptic transmission is that fusion of a transmitter-laden vesicle results in the near simultaneous activation of receptors clustered in the postsynaptic membrane. In a manner indistinguishable from vesicular release at neuronal synapses, AMPA receptors in $NG2^+$ cells are repeatedly exposed to high concentration transients of glutamate [11,17]. The resulting unitary events cause small (3–5 mV) depolarizations from their resting potential of ~ -100 mV, but exhibit pronounced frequency facilitation, similar to the Schaffer collateral-commissural fibers that populate this region. Indeed, stimulation of CA3 pyramidal neurons triggered bursts of inward currents in $NG2^+$ cells in area CA1, and electron microscopic analysis revealed that one Schaffer collateral axon formed synaptic junctions with both the dendrite of a CA1 pyramidal neuron and an $NG2^+$ cell process [11].

Neuronal excitability is tightly controlled by GABAergic feedforward and feedback inhibitory pathways. Cultured oligodendrocyte progenitor cells and glial progenitors *in situ* also express ionotropic $GABA_A$ receptors [18–20], suggesting that $NG2^+$ cells are also a target of the dense network of GABAergic interneurons that reside in the hippocampus. Indeed, electrical stimulation in the vicinity of CA1 $NG2^+$ cells in the presence of an AMPA receptor antagonist induced currents that were completely blocked by the $GABA_A$ receptor antagonist gabazine [12]. Furthermore, spontaneous miniature inhibitory postsynaptic currents (mIPSCs) were observed in $NG2^+$ cells in the presence of tetrodotoxin (TTX), which is indicative of vesicular release. The conclusion that $NG2^+$ cells also form synaptic junctions with interneurons was supported by the electron microscopic identification of pleomorphic vesicles (see glossary), which typically contain GABA, in nerve endings directly adjacent to membranes of physiologically identified $NG2^+$ cells. However, detailed structural analysis of these junctions will require immunolocalization of $GABA_A$ receptors and vesicular GABA transporter (VGAT).

Although GABA triggers a depolarization in developing neurons because of their high level of intracellular Cl^-, by the third postnatal week GABA elicits a membrane hyperpolarization in pyramidal neurons of the hippocampus; however, $NG2^+$ cells at this age were found to maintain a high intracellular Cl^- concentration, which forced the Cl^- equilibrium potential to reside at -43 mV. Given that the resting membrane potential of $NG2^+$ cells is close to the potassium equilibrium potential (~ -100mV), GABAergic signaling should depolarize these cells. Indeed, spontaneous GABAergic events depolarized $NG2^+$ cells by up to 5 mV. Although this modest depolarization was not sufficient to activate voltage-gated Ca^{2+} channels, $GABA_A$ receptor activation partially inhibited AMPA receptor currents through shunting (see glossary) and by an as yet undefined mechanism, suggesting that GABA exerts a net inhibitory effect in these glia as it does in neurons [12].

Recent studies have taken advantage of transgenic mice that express enhanced green fluorescent protein (EGFP) under the control of a 2.2 kb fragment of the human GFAP promoter [21] to visualize and examine the physiological properties of putative astrocytes in the mouse hippocampus [17,22]. Two populations of cells were distinguished in these animals on the basis of intensity of EGFP fluorescence. One group expressed EGFP at a high level, had a low membrane resistance (less than 10 MΩ), and expressed glutamate transporters, which were termed GluT cells; these cells represent typical

Figure 1



The distribution of NG2$^+$ cells in the brain. **(a)** Fluorescence image showing NG2$^+$ cells (yellow, NG2 immunoreactivity) and neuronal dendrites (blue, Map2 immunoreactivity) in a coronal slice of brain from a one month old mouse. Scale = 100 μm. **(b)** Higher magnification image of the area outlined in red in (a) illustrating the fine processes of NG2$^+$ cells that extend into the surrounding neuropil. Scale = 20 μm. Abbreviations: CC, corpus callosum; Ctx, cortex; Stm, striatum.

astrocytes. The second group of cells expressed EGFP at a lower level, had a higher membrane resistance (>50 MΩ), and expressed AMPA receptors [22]. These cells were termed GluR cells to distinguish them from astrocytes. The membrane properties of GluR cells were indistinguishable from those reported for NG2$^+$ cells, and in a latter study all GluR cells were found by posthoc immunostaining to express AN2, the mouse homolog of NG2 [23], indicating that GluR cells are NG2$^+$ cells. Indeed, in a subsequent report, Jabs and co-workers [17] showed that spontaneous and evoked AMPA and $GABA_A$ receptor currents could be recorded from GluR cells in the juvenile mouse hippocampus, confirming observations made in the rat hippocampus [11,12]. The fact that NG2$^+$ cells express EGFP in GFAP–EGFP mice is consistent with previous reports showing that GFAP mRNA, but not protein, is present in NG2$^+$ cells acutely isolated from the hippocampus [23].

Plasticity of neuron–NG2$^+$ cell synapses in the hippocampus

Synapses formed between Schaffer collaterals and CA1 pyramidal neurons in the hippocampus have served as a model for understanding the mechanisms responsible for use-dependent long term changes in synaptic strength. A recent study addressed whether glutamatergic synapses between Schaffer collaterals and NG2$^+$ cells in area CA1 of the rat hippocampus also exhibited activity-dependent plasticity [24•]. One might not expect these neuro–glia synapses to exhibit the same forms of plasticity as neuronal synapses, as NG2$^+$ cells express few, if any, N-methyl-D-aspartate (NMDA) receptors; EPSCs recorded from these cells are completely blocked by AMPA receptor antagonists [11,24•]. Remarkably, however, theta burst stimulation (TBS, see glossary) of the Schaffer collateral–commissural fibers resulted in a persistent increase in the amplitude of EPSCs in NG2$^+$ cells, with the amount of potentiation (~50%) similar to that observed in CA1 pyramidal neurons during long-term potentiation (LTP). This form of glial LTP, or gLTP as the authors define it, was not dependent on NMDA receptor activation, rather it was dependent on AMPA receptors, as gLTP was inhibited by application of philanthotoxin-433, a toxin which specifically blocks Ca^{2+} permeable AMPA receptors. In both pyramidal neurons and interneurons, activity-dependent increases in EPSC amplitude result from the insertion of additional Ca^{2+}-impermeable AMPA receptors into the postsynaptic membrane [25,26]. By contrast, plasticity of glutamatergic synapses in NG2$^+$ cells appears to result from the addition of Ca^{2+}-permeable receptors, as the EPSC rectification ratio (see glossary) increased following gLTP. Thus, increased activity in these afferents would be expected to enable greater Ca^{2+} influx by these same inputs. Although it is not yet clear how this positive feedback loop is constrained, TBS in the presence of an intracellular Ca^{2+} chelator resulted in synaptic depression, suggesting that Ca^{2+}-independent mechanisms of plasticity might also exist in these cells.

Intriguing new data from human tissue suggest that AMPA receptor expression in NG2$^+$ cells is also regulated in disease. Steinhauser and co-workers [27] examined hippocampal tissue from epilepsy patients that suffered from lesions in the temporal lobe that spared the hippocampus. Both GluR (NG2$^+$ cells) and GluT cells (astrocytes) were present in this putatively normal tissue. However, hippocampal tissue resected from patients suffering from Ammon's horn sclerosis (AHS, see Glossary), in which astrogliosis (see glossary) and neuronal loss

were observed in the hippocampus, contained GluR but no GluT cells, suggesting that seizure activity results in a selective loss of astrocytes. Furthermore, a comparison of GluR cells in normal and AHS tissue revealed that the expression of the Flip isoform of GluR1 (see glossary) was increased in GluR cells in AHS tissue, a change that slows the desensitization of AMPA receptors [27]. Because the decay of EPSCs is accelerated by desensitization, these changes might also lead to greater Ca^{2+} influx with each unitary event. Further work will be needed to determine whether this change in receptor expression is a consequence of the sclerosis and whether it contributes to human disease.

In summary, $NG2^+$ cells adapt to changes in hippocampal network activity by altering the number and properties of AMPA receptors that they express, and such changes are likely to result in enhanced Ca^{2+} influx into the processes of these glial cells.

$NG2^+$ cells in different brain regions receive synaptic input

These studies of $NG2^+$ cells in the hippocampus raise the question of whether rapid synaptic signaling is a conserved property of this class of glial cells. $NG2^+$ cells are widely distributed in the mature CNS; however, physiological studies of these cells in regions other than the hippocampus have been hampered by the inability to distinguish these cells from surrounding neurons and glia. Two recent studies overcame this limitation by using transgenic mice that express EGFP under the control of oligodendrocyte-associated promoters.

In the first study, mice that express EGFP under the control of a 3.7 kb fragment of the promoter for the oligodendrocyte lineage marker 2′–3′-cyclic nucleotide 3′-phosphodiesterase (CNPase) [28] were used to compare the properties of $NG2^+$ cells in highly myelinated regions, such as the subcortical white matter, and regions with scarce myelination, such as the cerebral cortex [29]. Chittajallu and co-workers [29] found that white matter $EGFP^+$ cells had a higher membrane resistance and a more depolarized resting membrane potential than cortical $EGFP^+$ cells. Furthermore, in the cortex about 30% of the $NG2^+$ cells in these 5–10 day old mice exhibited TTX-sensitive sodium spikes upon depolarization, and at this young age AMPA receptor mediated currents were observed under pharmacological conditions that favor spontaneous vesicle release. Although the high threshold for spike initiation and the negative resting potential exhibited by these cells make it unlikely that these cells fire action potentials, the observed differences between $NG2^+$ cells from white matter and those from the cortex might indicate that $NG2^+$ cells in different brain regions have distinct functions. Previous studies have shown that OPCs (putative $NG2^+$ cells) have the potential to develop into neurons *in vitro* [6], and recent work by Gallo and co-workers [30,31] showed that $NG2^+$ cells injected into the lateral ventricles of early postnatal mice can develop into inhibitory neurons in the olfactory bulb and hippocampus. Therefore, the expression of a greater number of Na^+ channels by some cortical $NG2^+$ cells might indicate a transition towards a neuronal phenotype. However, $NG2^+$ cells appear to lose the ability to spike as K^+ channel expression increases during the second and third postnatal weeks, and the extent of synaptic activity was not correlated with the presence of a Na^+ current [29]. Ultimately, a comprehensive analysis of the developmental potential of $NG2^+$ cells *in vivo* will require the development of transgenic lines that express Cre recombinase under the control of the NG2 or PDGFαR promoters.

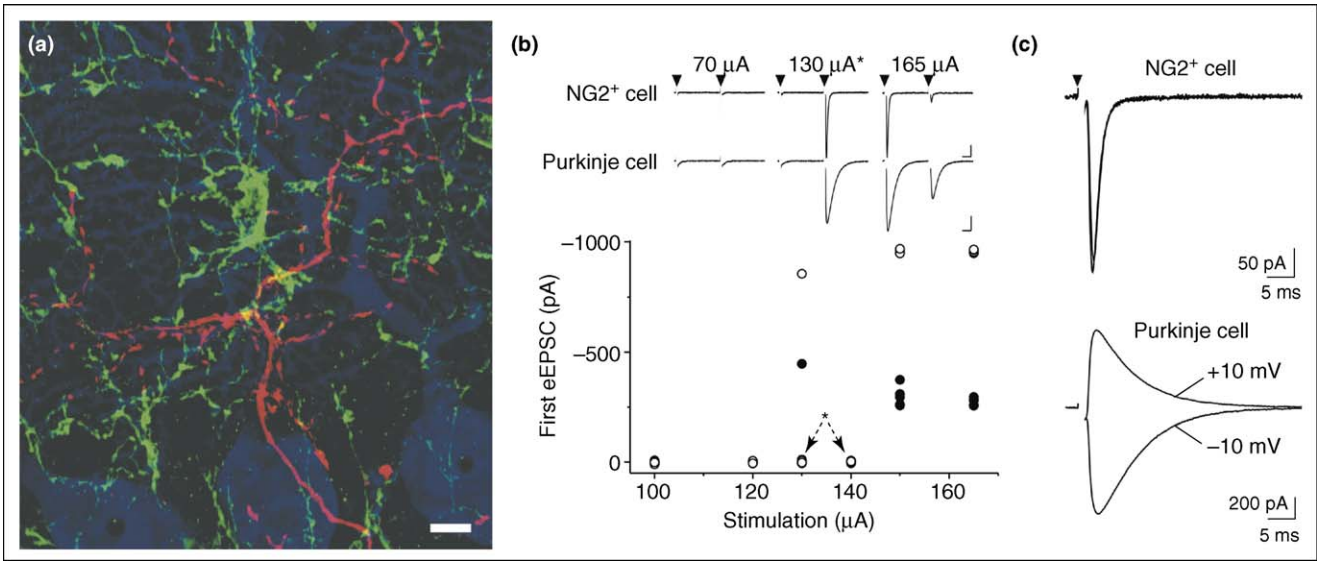
In the second study, $NG2^+$ cells were visualized using mice that express EGFP under the control of a 10.6 kb fragment of the proteolipid protein (PLP) promoter. In these PLP–EGFP mice, both oligodendrocyte progenitors ($NG2^+$, $PDGF\alpha R^+$) and mature oligodendrocytes express EGFP [32]. To distinguish $NG2^+$ cells from oligodendrocytes, the authors took advantage of the fact that the oligodendrocytes are absent from molecular layer of the cerebellum. In terms of synaptic organization, the cerebellum is one of the most architecturally simple areas of the central nervous system (CNS), making it an ideal place to study neuro–glia cell interactions. In the molecular layer, parallel fibers and climbing fibers (CFs) make excitatory synapses with the dendrites of Purkinje cells (PCs). Lin and co-workers [13••] observed that focal electrical stimulation induced large (>1nA) EPSCs in molecular layer $NG2^+$ cells. These responses exhibited the same all-or-none behavior and prominent paired-pulse depression as CF responses in PCs, and simultaneous recordings from PCs and $NG2^+$ cells revealed that a single CF can form synapses with both cell types (Figure 2). Quantal analysis revealed that each CF response was produced by the release of as many as 50 vesicles; if release at each CF–$NG2^+$ cell synapse is uniquantal, these data indicate that one CF forms many synapses with each $NG2^+$ cell. Furthermore, at an age when most PCs are innervated by a single CF, many $NG2^+$ cells were innervated by multiple CFs. Therefore, $NG2^+$ cells appear to be a previously unrecognized element in the cerebellar network.

Taken together, these recent studies indicate that $NG2^+$ cells in the cerebellum and cortex also form direct synapses with glutamatergic neurons, suggesting that this mode of signaling is a conserved property of $NG2^+$ cells in gray matter.

Ectopic glutamate release onto astroglia

Astroglia (astrocytes and Bergmann glia) extend thin processes that ensheath excitatory synapses, delivering glutamate transporters to sites of release and creating barriers to limit diffusion between adjacent synapses. Imaging studies have shown that mGluR-mediated

Figure 2



Climbing fiber input to NG2$^+$ cells in the cerebellum. The molecular layer of the cerebellum contains two distinct classes of glial cells, Bergmann glia and a group of stellate shaped glial cells that express the NG2 proteoglycan, termed NG2$^+$ cells. **(a)** Fluorescence image showing a climbing fiber (red, Alexa 488) ramifying within the molecular layer among the processes of NG2$^+$ cells (green, NG2 immunoreactivity) and Purkinje cells (blue, Calbindin immunoreactivity). Scale = 10 µm. Reprinted with permission from *Nature Reviews Neuroscience* [41]. **(b)** Simultaneous recording from an NG2$^+$ cell and a Purkinje cell. Lower panel: plot of first evoked EPSCs (eEPSC) in a paired-pulse protocol (interval: 50 ms) against stimulation intensity from this pair (NG2$^+$ cell, $V_M = -80$ mV; Purkinje cell, $V_M = -10$ mV). Closed symbols represent data from the NG2$^+$ cell and open symbols from the Purkinje cell. The asterisk and arrows indicate the intensity when the first stimulation failed yet the second stimulation succeeded in eliciting EPSCs in both cells. Upper panel: average traces from this pair of cells at different stimulation intensities. Scale bars: 10 ms/50 pA for NG2$^+$ cell EPSCs, 10 ms/200 pA for Purkinje cell EPSCs. **(c)** Holding the Purkinje cell at different membrane potentials (lower panel) did not affect NG2$^+$ cell EPSCs (2 traces, upper panel), indicating that the response in the NG2$^+$ cell is not due to the response in the Purkinje cell. These results indicate that there are two distinct targets of the olivocerebellar projection — Purkinje neurons and NG2$^+$ glial cells — and suggest that a primary goal of climbing fiber axons is to influence the behavior of these abundant glial cells. Arrowheads in (b) and (c) indicate when the stimulation was delivered. Reprinted with permission from Neuron [13••], with permission from Elsevier.

Ca^{2+} rises can be induced in astrocytes by the synaptic release of glutamate [33–35], and overt AMPA receptor currents have been observed in Bergmann glia (BG) [34–36]. Although it was previously thought that glutamate encountered these perisynaptic membranes as it attempted to escape from the cleft, the discovery of miniature EPSCs in BG indicates that receptor activation in astroglia can also arise from fusion of vesicles outside active zones, at sites directly opposite ensheathing membranes [36]. This ectopic release of vesicles also results in rapid, brief activation of AMPA receptors, so how does this mode of signaling differ from that observed in NG2$^+$ cells? Because both NG2$^+$ cells and BG receive input from CFs, it is possible to compare signaling in these two types of glia directly. A key finding made by Matsui and Jahr [36] was that unitary events in BG were not simultaneously observed in PCs, suggesting that vesicles can fuse at different locations in the nerve terminal, and that there is little crosstalk, at least as far as AMPA receptors are concerned, between these compartments. CF-mediated AMPA receptor currents in BG were also more sensitive to reductions in Ca^{2+} influx than were CF EPSCs in PCs [37], suggesting that vesicles responsible for BG events are further from the Ca^{2+} channel rich active zones. Consistent with this hypothesis, AMPA currents in BG were also more sensitive than were CF–PC responses to inhibition of N-type Ca^{2+} channels, channels that are thought to be more widely distributed in nerve terminals. By comparison, CF responses recorded from NG2$^+$ cells exhibited the same degree of sensitivity as EPSCs in PCs to N-type Ca^{2+} channel inhibition [13••]. Another difference between the two modes of signaling occurs as a consequence of the anatomy of these neuro–glia junctions. Whereas BG ensheath CF–PC synapses, NG2$^+$ cells appear to be a direct target of axon collaterals. As a result, when the apparent affinity of AMPA receptors is increased with cyclothiazide (CTZ), the amplitude of CF–BG EPSCs is increased more than eightfold, as these receptors become responsive to glutamate that spills out of the cleft [38]. By contrast, CF EPSCs in NG2$^+$ cells are increased only about twofold by CTZ [13••], similar to the increase observed at neuronal synapses. Finally, synaptic currents can be elicited repeatedly in NG2$^+$ cells for hours, suggesting a robust mechanism for vesicle replenishment is in place at these synapses. By contrast, CF responses in BG fatigue rapidly, and it is not unusual to

obtain responses to only the first few stimuli that are applied, suggesting that ectopic pools are subject to more rapid depletion. These findings support a general framework whereby $NG2^+$ cells are a direct target of afferent inputs, whereas astroglia are activated as a consequence of their tight association with neuronal synapses.

Conclusions

Recent studies indicate that a widely distributed class of glial progenitors referred to as $NG2^+$ cells engage in rapid signaling with surrounding neurons through direct synapses. This mode of communication has been found in different brain regions, including the hippocampus, cortex and cerebellum, and is present in both the developing and the mature CNS. These neuro–glia synapses initiate Ca^{2+} signaling within $NG2^+$ cells, and the strength of these inputs, and therefore Ca^{2+} influx, can be modified by neuronal activity. These findings raise many new questions about the role of these enigmatic glial cells in the mature brain and the degree to which their behavior is modified by activity at these synapses. Among the most pressing questions that remain are the functional significance of this rapid synaptic signaling and the developmental potential of this vast population of proliferating cells. *In vitro* studies indicate that receptor activation can alter the development of these progenitors [39,40]; however, it remains to be determined whether synaptic signaling plays a permissive or instructive role in $NG2^+$ cell development *in vivo*. Answers to these questions might reveal new pathways for modulation of neuronal activity, and present new approaches for both preventing myelin damage and accelerating remyelination following ischemic injury or demyelinating diseases.

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