Brain Research

Research Report

Electrophysiological properties of NG2$^+$ cells: Matching physiological studies with gene expression profiles

CrossMark

Valerie A. Larson[a], Ye Zhang[b], Dwight E. Bergles[a],*

[a]Solomon H. Snyder Department of Neuroscience, Johns Hopkins University School of Medicine, Baltimore, MD 21205, USA
[b]Department of Neurobiology, Stanford University School of Medicine, Stanford, CA 94305, USA

ARTICLE INFO

ABSTRACT

NG2$^+$ glial cells are a dynamic population of non-neuronal cells that give rise to myelinating oligodendrocytes in the central nervous system. These cells express numerous ion channels and neurotransmitter receptors, which endow them with a complex electrophysiological profile that is unique among glial cells. Despite extensive analysis of the electrophysiological properties of these cells, relatively little was known about the molecular identity of the channels and receptors that they express. The generation of new RNA-Seq datasets for NG2$^+$ cells has provided the means to explore how distinct genes contribute to the physiological properties of these progenitors. In this review, we systematically compare the results obtained through RNA-Seq transcriptional analysis of purified NG2$^+$ cells to previous physiological and molecular studies of these cells to define the complement of ion channels and neurotransmitter receptors expressed by NG2$^+$ cells in the mammalian brain and discuss the potential significance of the unique physiological properties of these cells.

This article is part of a Special Issue entitled SI:NG2-glia(Invited only).

1. Introduction

NG2$^+$ cells comprise a widely distributed, dynamic population of precursor cells that remain abundant in the adult central nervous system (CNS). These cells have the ability to mature into oligodendrocytes and are critical for providing new oligodendrocytes to allow remyelination of axons following injury and in diseases such as multiple sclerosis, and are often referred to as oligodendrocyte precursor cells (OPCs). However, fate tracing studies indicate that most of these cells in the adult CNS do not differentiate into oligodendrocytes and these cells can be found in regions devoid of myelin, suggesting that they have other, as yet undefined, functions; for these reasons, they are termed NG2$^+$ cells in

*Correspondence to: Johns Hopkins University School of Medicine, Solomon H. Snyder Department of Neuroscience, Wood Basic Science Building 1003, 725 N. Wolfe Street, Baltimore, MD 21205, USA. Fax: +1 410 614 6249.
E-mail address: dbergles@jhmi.edu (D.E. Bergles).

this review. In mice, as well as humans, the generation of oligodendrocytes and the myelination of axons occurs over a protracted time period, beginning in early postnatal life and continuing well into adulthood (Waly et al., 2014). The extended period of myelination must be considered when discussing the physiological properties and behavior of NG2+ cells, as all physiological studies have been performed during this time. In such a dynamic cell population, time-, region-, and even individual cell-specific variability is to be expected, depending on the cell cycle stage, differentiation stage, and molecular and cellular milieu. Nevertheless, the numerous electrophysiological studies of NG2+ cells have provided critical insight into the conserved properties of these cells.

NG2+ cells are a unique glial population in that they display many characteristics typically associated with neurons. These include the expression of 'neuronal' genes, such as voltage-gated ion channels and ligand-gated ionotropic neurotransmitter receptors. Expression of these genes gives them a complex electrophysiological profile, in contrast to the linear profile of astrocytes and mature oligodendrocytes. Historically, this distinction has led to these cells being referred to as "complex astrocytes" or "complex glia" in the literature (Matyash and Kettenmann, 2010; Steinhäuser et al., 1994). Another unique feature of NG2+ cells is that they are the only glial cell type known to be capable of receiving synaptic connections from neurons (Bergles et al., 2000; Jabs et al., 2005; Lin and Bergles, 2004). At one time it was hypothesized that these cells could even differentiate into neurons (Aguirre and Gallo, 2004; Belachew et al., 2003; Goldman, 2003), although more recent studies have demonstrated that they are restricted to glial fates in vivo (see Akiko Nischiyama's chapter in this issue).

Much of our knowledge of the properties of NG2+ cells comes from whole-cell patch clamp recordings performed on cultured oligodendrocyte precursor cells, called O-2A cells due to their presumed ability to generate oligodendrocytes and type II astrocytes, and on NG2+ cells in acute brain slices. Physiological and pharmacological evidence has pointed to the expression of a vast array of channels and receptors in the membranes of these cells. Specific antagonists, RT-PCR, Western blot, and immunohistochemistry have provided some insight the molecular identity of the channels and receptors, but until recently there had been no systematic effort to catalog channel and receptor expression by NG2+ cells.

In recent years, two studies have used microarrays and RNA-Seq to systematically assay gene expression in a variety of cell types, including NG2+ cells (Cahoy et al., 2008; Zhang et al., 2014). The latter study used RNA-Seq to assess transcription levels of >22,000 genes within neurons, astrocytes, oligodendrocytes, NG2+ cells, microglia, endothelial cells, and pericytes (Zhang et al., 2014). NG2+ cells were harvested from P17 mouse cortex and depleted of microglia and endothelial cells by immunopanning on BSL1- and anti-CD45 antibody-coated plates. The remaining cells underwent immunopanning using an antibody specific for PDGFRα, a marker specific for NG2+ cells. This process resulted in a mostly pure NG2+ cell population, although the authors report that there is a remaining 5% contamination of microglia in the samples.

One obvious caveat to this data set is that it represents only a snapshot of gene expression at a particular time point (P17) and in one brain region (cortex), which cannot capture the full complexity of the population over time. Yet, it is still an invaluable resource to begin to understand the molecular basis for the physiological properties of NG2+ cells. Until now there has been no systematic effort to correlate these RNA-Seq findings with previous physiological and pharmacological studies. In this review, we will attempt to bring together these data sets and discuss the possible contributions of various channels and receptors to NG2+ cell behaviors. For consistency, the term "NG2+ cells" will be used when citing studies that examined OPCs/O-2A cells in vitro and in vivo, even if expression of the NG2 antigen was not confirmed. In cases where the oligodendroglial lineage of the cells was not established, terms such as "complex glia" or "glial progenitors" will be used, as appropriate.

The RNA-Seq transcriptome data is expressed in units of fragments per kilobase of transcript sequence per million mapped fragments (FPKM). Although a threshold of 0.1 FPKM was used to determine statistically significant expression (>99% confidence), due to space constraints, only more highly expressed channel and receptor genes will be considered in this review. A threshold of ≥ 2 FPKM was set for inclusion in summary tables. Genes with lower expression that have been identified in previous studies will be addressed in the text when appropriate. In addition, the discussion will be limited to plasma membrane ion channels and known neurotransmitter receptors.

2. Ion channels

2.1. Passive membrane properties

NG2+ cells in acute brain slices isolated from juvenile animals typically have a capacitance of 15–30 pF, a membrane resistance of 100–500 MΩ, and a stable resting membrane potential (RMP) between –80 and –100 mV (Clarke et al., 2012; De Biase et al., 2010; Haberlandt et al., 2011; Kukley et al., 2008; Lin and Bergles, 2002; Maldonado et al., 2013). This RMP is near the calculated equilibrium potential for K+ (E_K), suggesting that K+ channels account for the majority of the resting membrane conductance. The predominant K+ channels open at rest ('leak' channels) are the inward-rectifier Kir4.1 and two-pore (K_{2P}) K+ channels.

Kir4.1 is an inward-rectifying channel expressed by astrocytes, oligodendrocytes, and NG2+ cells that has been implicated in setting the RMP. This channel also plays a prominent role in the buffering of K+ by Müller glia in the retina and astrocytes in the brain (Butt and Kalsi, 2006; Chever et al., 2010; Djukic et al., 2007; Kofuji et al., 2000; Neusch et al., 2006, 2001). The RNA-Seq transcriptome database shows that Kir4.1 (Kcnj10) mRNA is expressed at high levels in NG2+ cells (OPCs), higher than any other K+ channel subunit (See Table 1), although only at approximately half the level observed in astrocytes (Zhang et al., 2014). This is consistent with data from Kir4.1-EGFP BAC-transgenic mice, in which EGFP intensity, which reflects promoter activity, was observed to be approximately three-fold higher in GFAP+

Table 1 – K⁺ channels.

Gene Name	Also known as	Expression level in NG2⁺ cells (FPKM)	Additional information (Gutman et al., 2005)
Kcnj10	Kir4.1	82.6	Inward rectifier
Kcnmb4	$K_{Ca}\beta_4$	45.5	BK (β subunit)
Kcnd2	K_v4.2	27.0	A-type
Kcna6	K_v1.6	24.1	Delayed rectifier
Kcnk1	K_{2P}1.1/ TWIK1	24.0	Inward rectifier (two pore)
Kcnd3	K_v4.3	22.3	A-type
Kcnk2	K_{2P}2.1/ TREK1	19.6	Inward rectifier (two pore)
Kcnq2	K_v7.2	10.9	Delayed rectifier
Kcnc3	K_v3.3	8.9	A-type
Kcnab2	$K_v\beta_2$	8.8	K_v (β subunit)
Kcnk10	K_{2P}10.1	7.9	Inward rectifier (two pore)
Kcnj16	Kir5.1	7.0	Inward rectifier
Kcnb1	K_v2.1	6.8	Delayed rectifier
Kcnma1	K_{Ca}1.1	6.3	Large conductance calcium-activated (BK)
Kcnh2	K_v11.1/ HERG	5.5	Inward rectifier
Kcnn1	K_{Ca}2.1	4.9	Small conductance calcium-activated (SK)
Kcna2	K_v1.2	4.8	Delayed rectifier
Kcnab3	$K_v\beta_3$	4.6	K_v (β subunit)
Kcne4	MiRP3	4.5	K_v (β subunit)
Kcnab1	$K_v\beta_1$	4.5	K_v (β subunit)
Kcnc1	K_v3.1	4.1	Delayed rectifier
Kcnh8	K_v12.1	4.1	Slowly activating/ inactivating voltage-gated channel
Kcnn2	K_{Ca}2.2	3.7	Small conductance calcium-activated (SK)
Kcna3	K_v1.3	3.6	Delayed rectifier
Kcnh3	K_v12.2	3.6	Voltage gated
Kcnj3	Kir3.1	3.4	Inward rectifier
Kcna1	K_v1.1	3.4	Delayed rectifier
Kcnh5	K_v10.2	3.1	Outward rectifier, noninactivating
Kcnd1	K_v4.1	2.9	A-type
Kcnq3	K_v7.3	2.8	Delayed rectifier
Kcnc4	K_v3.4	2.4	A-type
Kcnf1	K_v5.1	2.3	Modifier of K_v2 channels

astrocytes than in NG2⁺ cells (Tang et al., 2009). Kir4.1 subunits can form heteromeric channels with Kir5.1 (Kcnj16) subunits, resulting in channels with increased single-channel conductance and greater pH sensitivity (Butt and Kalsi, 2006; Hibino et al., 2004). mRNA for Kir5.1 is found at approximately two-fold higher levels in NG2⁺ cells than in astrocytes, perhaps indicating a relatively greater role for heteromeric channels in these cells (Zhang et al., 2014).

Kir4.1 mediates the inward currents observed in whole cell recordings when the membrane potential of NG2⁺ cells is hyperpolarized from the RMP (Fig. 1A–D). These inward currents are blocked by low concentrations of extracellular Ba^{2+}, an inhibitor of Kir channels, and are also specifically abolished in "complex glia" of Kir4.1 glia-specific conditional knockout (cKO) animals (Djukic et al., 2007). "Complex glia" in Kir4.1 cKO animals

are significantly depolarized to ∼ –40 mV, have increased membrane resistance (∼500 MΩ), and appear to be greatly reduced in number in the hippocampus. These observations, together with the severe disruption of myelin in Kir4.1 knockout animals (Djukic et al., 2007; Neusch et al., 2001), suggest an important role for Kir4.1 in the development, survival, or differentiation of NG2⁺ cells.

The membrane resistance of NG2⁺ cells remains low even in the presence of internal solution containing the K⁺ channel blockers Cs⁺ and tetraethylammonium (TEA) (D. Bergles, unpublished observations). This suggests the hypothesis that NG2⁺ cells must express additional 'leak' channels that are not sensitive to these blockers. Two-pore domain (K_{2P}) K⁺ channels fit this pharmacological profile (Feliciangeli et al., 2014), and several studies have characterized the expression of K_{2P} channels by passive astrocytes (Seifert et al., 2009; Zhou et al., 2009), but relatively little is known about the expression and functional contribution of these channels in NG2⁺ cells. A major road-block is the lack of good pharmacological tools to specifically target these channels (Feliciangeli et al., 2014). In a recent study, no change in NG2⁺ cell current properties was found in a TWIK1 knockout mouse, and no effect was observed on applying isoflurane, an activator of TREK1 (Maldonado et al., 2013). Yet, the RNA-Seq transcriptome database has identified several K_{2P} isoforms, notably TWIK1 (Kcnk1), TREK1 (Kcnk2), and Kcnk10, as highly-expressed in NG2⁺ cells (See Table 1) (Zhang et al., 2014). One possible explanation for the discrepancy between mRNA expression data and physiological data is that channels may not be heavily trafficked to the cell membrane, despite high levels of transcription and/or translation. This was found to be the case for TWIK1 in passive astrocytes, where the channel is largely sequestered in the intracellular compartment (Feliciangeli et al., 2010; Wang et al., 2013). Better pharmacological and genetic tools will enable a more thorough investigation of the contribution of K_{2P} channels to the passive conductance of NG2⁺ cells.

In addition to their complement of K⁺ 'leak' channels, the RNA-Seq transcriptome database indicates that NG2⁺ cells express the Na⁺ leak channel Nalcn (See Table 2). This expression is at approximately the same level as that in neurons, and is five-fold higher than that of mature oligodendrocytes (Zhang et al., 2014). This slight baseline permeability to Na⁺ is another example of NG2⁺ cells displaying typically neuronal properties. But, due to the hyperpolarized RMP of these cells, it is clear that the K⁺ leak conductance dominates to a greater extent than in neurons.

It should be noted that NG2⁺ cell membrane properties are not constant during postnatal development. It has been shown that NG2⁺ cells undergo a dramatic change in their current–voltage relationship over the course of development from the first to the fifth postnatal week, transitioning from outward rectification to an increasingly linear phenotype (De Biase et al., 2010; Maldonado et al., 2013). This change has been attributed to an increase in Kir4.1 expression, as the Ba^{2+}-sensitivity of the currents increases over the same time period (Maldonado et al., 2013). These findings are in agreement with earlier studies of the changing electrophysiological properties of unidentified "complex glia" in hippocampus over time (Kressin et al., 1995; Zhou et al., 2006), as well as
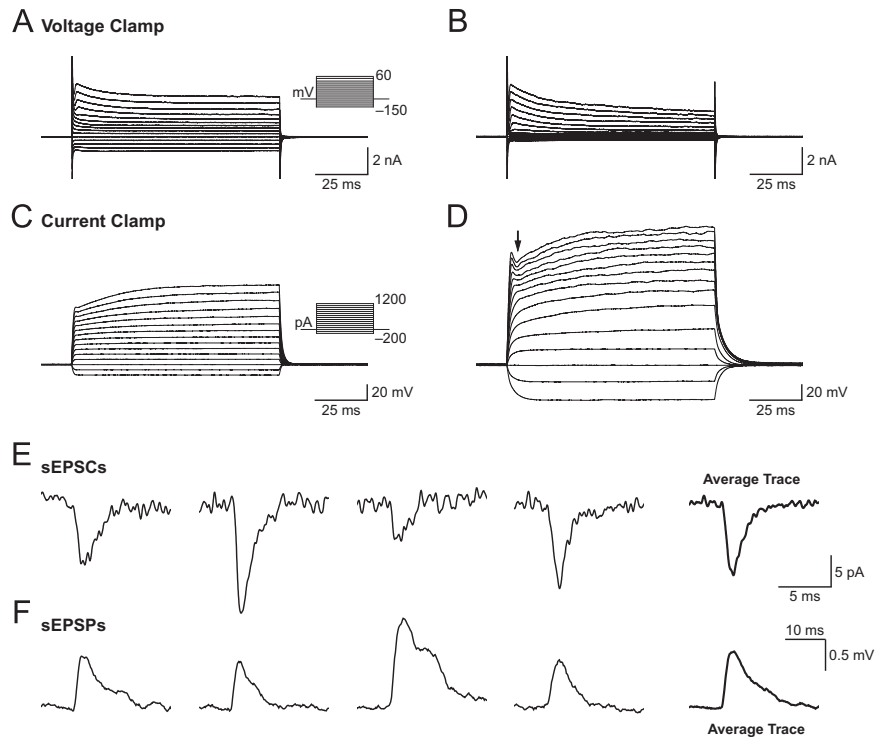
Fig. 1 – Membrane properties of NG2+cells and response to glutamatergic synaptic input. (A, B) Voltage clamp recordings from hippocampal NG2+ cells in acute brain slices isolated from a 5 week-old *PDGFRα-CreER; Rosa-CAG-EGFP(RCE)* mouse showing activation of voltage gated currents upon application of hyperpolarizing and depolarizing voltage steps from a holding potential of –90 mV. Voltage step induced artifacts at the beginning of the traces have been truncated for clarity. Cell in A: $C_m=20.44$ pF, $R_m=54.2$ MΩ, $R_a=18.5$ MΩ; Cell in B: $C_m=25.94$ pF, $R_m=217.1$ MΩ, $R_a=13.5$ MΩ. (C, D) Current clamp recordings from the same cell as in A and B, respectively, showing the membrane potential changes induced by current injections from a resting membrane potential of –90.2 mV (C) and – 91.9 mV (D). Arrow in D highlights inflection induced by rapid inactivation of A-type K+ channels. (E) Examples of spontaneous EPSCs (sEPSCs) recorded from an NG2+ cell in the corpus callosum of the same animal. Average trace is of the four events shown. (F) Examples of spontaneous EPSPs (sEPSPs) from the same cell as in (E), highlighting the small depolarization produced by these synaptic currents. Average trace is of the four events shown.

Table 2 – Na+ channels.			
Gene Name	Also known as	Expression level in NG2+ cells (FPKM)	Additional information (Catterall et al., 2005a)
Nalcn	Nacln	19.3	Sodium leak channel
Scn1b	Na$_v$β$_1$	14.5	Voltage-gated (β subunit)
Scn3b	Na$_v$β$_3$	12.9	Voltage-gated (β subunit)
Scn3a	Na$_v$1.3	11.4	Voltage-gated (TTX sensitive)
Scn2a1	Na$_v$1.2	7.6	Voltage-gated (TTX sensitive)
Scn8a	Na$_v$1.8	5.0	Voltage-gated (TTX resistant)
Scn1a	Na$_v$1.1	4.9	Voltage-gated (TTX sensitive)
Scn2b	Na$_v$β$_2$	4.8	Voltage-gated (β subunit)

studies showing the broad upregulation of Kir4.1 in the CNS during postnatal development using sqRT-PCR, Western blot, and immunohistochemistry (Gupta and Kanungo, 2013; Kalsi et al., 2004; Nwaobi et al., 2014; Seifert et al., 2009). Such variability in channel expression must be taken into account when considering the results of the available RNA-Seq transcriptome database, which only captures NG2+ cells at one time in development (P17).

There is also regional variability in the passive membrane properties of NG2+ cells. In P5-P10 animals, a comparison of cortical and callosal NG2+ cells showed that white matter NG2+ cells have smaller capacitances (8 pF vs. 40 pF), higher membrane resistances (>2 GΩ vs. 500 MΩ), and a less negative RMP (–70 mV vs. –87 mV) than cells in gray matter (Chittajallu et al., 2004; De Biase et al., 2010). White matter NG2+ cells also have much smaller Cs+-sensitive Kir currents

than gray matter NG2$^+$ cells at this age. Although there are differences between white and gray matter early in postnatal development, these changes become less distinct with age as NG2$^+$ cells dramatically increase their membrane conductance (De Biase et al., 2010).

2.2. Voltage-gated channels and excitability

2.2.1. Voltage-gated K$^+$ channels

Upon depolarization, NG2$^+$ cells display a non-linear current profile that is characteristic of A-type (K_A) and delayed-rectifier (K_{DR}) K$^+$ channels (Fig. 1A–D). These currents have been extensively characterized in culture and brain slice preparations (Barres et al., 1990; Berger et al., 1991; Borges et al., 1995; Kettenmann et al., 1991; Sontheimer et al., 1989; Williamson et al., 1997). A-type channels exhibit rapid activation and inactivation kinetics, contributing to the initial 'peak' depolarization; inactivation is so rapid that is gives the impression of a spike on the rising phase of the membrane response (see Fig. 1D). Delayed-rectifiers, which activate more slowly and do not inactivate, contribute to the sustained 'steady-state' current. The delayed-rectifier current is blocked by internal Cs$^+$ and external TEA, and the A-type current is blocked by 4-aminopyridine (4-AP) and internal Cs$^+$ (Barres et al., 1990; Borges et al., 1995; Kettenmann et al., 1991; Sontheimer et al., 1989; Williamson et al., 1997). The relative proportions of the two current components vary by region. When compared to cortical NG2$^+$ cells, white matter NG2$^+$ cells in P5-P10 mice have higher I_{KDR} current densities, while I_{KA} density is the same, resulting in a higher I_{KDR}/I_{KA} ratio (Chittajallu et al., 2004).

The precise molecular identity of the channels responsible for these currents is not known. Most studies to date have focused on the Shaker family of K$^+$ channels, K_v1.1–1.6. RT-PCR and immunocytochemical localization in cultured NG2$^+$ cells have shown expression of K_v1.2, K_v1.4, K_v1.5, and K_v1.6 mRNA and protein, with K_v1.5 being the highest expressed (Attali et al., 1997). Another study using single-cell RT-PCR found that individual cultured progenitors can express varying combinations of all six Shaker type channels, with K_v1.2, K_v1.5, and K_v1.6 being the most commonly expressed. However, only K_v1.4, K_v1.5, and K_v1.6 were found at the protein level using immunocytochemistry (Schmidt et al., 1999). A third study also using immunocytochemistry found expression of K_v1.3 through K_v1.6 protein in cultured progenitors (Chittajallu et al., 2002).

One consistent trend across multiple studies is that specific inhibition or knockdown of individual channel subunits (with the exception of K_v1.5 knockdown (Attali et al., 1997) and a partial effect of a toxin specific to K_v1.3 (Chittajallu et al., 2002)) has only minimal effects on the observed current properties of cultured NG2$^+$ cells (Attali et al., 1997; Chittajallu et al., 2002; Schmidt et al., 1999). These observations, along with the heterogeneity of expression observed at the mRNA and protein levels, suggests that NG2$^+$ cells express an assortment of heteromeric K$^+$ channels that, in most cases, can compensate for missing individual subunits to maintain the overall current profile. Another important trend is that mRNA and protein levels of K_v channel subunits appear to be regulated independently, leading to disparities between mRNA and protein expression even within the same study (Attali et al., 1997; Chittajallu et al., 2002; Schmidt et al., 1999). This reinforces the need to exercise caution when using mRNA levels to make inferences about the abundance of membrane K$^+$ channels.

With this caveat in mind, the RNA-Seq transcriptome database provides the most comprehensive analysis yet of K$^+$ channel expression in NG2$^+$ cells. Consistent with previous studies, mRNA for the delayed rectifier K_v1.6 is highly expressed (See Table 1). mRNA for the other previously identified Shaker type delayed rectifiers, however, is expressed only at lower levels: K_v1.2 and K_v1.3 are expressed at low but significant levels (See Table 1), while K_v1.5 is expressed at very low levels (0.8 FPKM). In addition, mRNA for several non-Shaker type delayed rectifier channels, K_v7.2 and K_v2.1, are expressed at high levels. K_v7.2 has been previously localized by RT-PCR and immunohistochemistry to at least a subset of cortical NG2$^+$ cells (Wang et al., 2011). K_v2.1 has been found in many subtypes of neurons (Du et al., 1998; Trimmer, 1991) and in Schwann cells (Sobko et al., 1998), but to our knowledge has never been reported in NG2$^+$ cells.

K_v1.4, the only Shaker type channel to display A-type properties, has only low expression in NG2$^+$ cells in the database (0.7 FPKM). Other A-type channel subunits that are highly expressed are K_v4.2, K_v4.3, and Kv3.3. K_v4.2 and 4.3 have been found in neurons (Tsaur et al., 1997) and cultured astrocytes (Bekar et al., 2005), but never previously in oligodendrocyte lineage cells. Kv3.3 has been found in neurons, particularly in the cerebellum and brainstem (Chang et al., 2007), but not in glial cells. Of the K_v beta subunits, $K_v\beta_2$ was the most highly expressed, followed by $K_v\beta_3$ and $K_v\beta_1$.

The large conductance Ca^{2+}-activated [BK] channel K_{Ca}1.1, along with its β_4 subunit, is highly expressed in NG2$^+$ cells in the database (See Table 1). This confirms previous findings that BK channels, which are activated by both voltage and intracellular Ca^{2+}, are expressed in cultured NG2$^+$ cells (Buttigieg et al., 2011). There has also been some evidence of small-conductance Ca^{2+}-activated [SK] channel expression in cultured NG2$^+$ cells (Barres et al., 1990; Sontheimer et al., 1989); in the database, the SK channel subunits K_{Ca}2.1 and K_{Ca}2.2 are expressed at low but significant levels.

Voltage-gated K$^+$ channel expression by NG2$^+$ cells is known to play an important role in regulating cell behavior. K_v1.3 is upregulated during the G_1 phase of the cell cycle in NG2$^+$ cells, and blockade of this channel with specific toxins prevents the cells from entering the G_1/S transition (Chittajallu et al., 2002). Conversely, overexpression of K_v1.3 or K_v1.4 promotes the proliferation of cultured NG2$^+$ cells in the absence of mitogens, while overexpression of K_v1.6 inhibits proliferation in the presence of mitogens (Vautier et al., 2004). Knockdown and overexpression of K_v1.5 have not produced any effects on NG2$^+$ cell proliferation (Attali et al., 1997; Vautier et al., 2004). Differentiation of cultured NG2$^+$ cells into oligodendrocytes is not significantly affected by overexpression of K_v channel subunits, demonstrating that proliferation and differentiation of these cells can be regulated independently (Vautier et al., 2004).

2.2.2. Voltage-gated Na^+ channels

Like neurons, $NG2^+$ cells also express tetrodotoxin (TTX)-sensitive voltage-gated Na^+ (Na_v) channels (Barres et al., 1990; Berger et al., 1992a; Bergles et al., 2000; Borges et al., 1995; Maldonado et al., 2011; Sontheimer et al., 1989; Williamson et al., 1997). The expression density of these channels varies by region in the brain (Chittajallu et al., 2004), and decreases as $NG2^+$ cells differentiate into oligodendrocytes (De Biase et al., 2010; Sontheimer et al., 1989). Expression of Na_v channels together with K_v channels, in principle, endows $NG2^+$ cells with the necessary machinery to generate action potentials. Indeed, it has been observed that, in early post-natal animals, injection of depolarizing current into $NG2^+$ cells can result in spikes, somewhat similar to action potentials (Chittajallu et al., 2004; De Biase et al., 2010; Káradóttir et al., 2008; Tong et al., 2009). However, these spikes have a high threshold for initiation, a smaller amplitude, slower kinetics, and are less stereotyped than neuronal action potentials. $NG2^+$ cells also exhibit a limited ability to sustain repetitive spiking in response to tonic depolarizing current injection, with most cells exhibiting a single spike (Chittajallu et al., 2004; De Biase et al., 2010) (but see (Káradóttir et al., 2008)). The physiological relevance of this phenomenon is unclear, as synaptic inputs produce only minimal depolarization of $NG2^+$ cells (see Fig. 1E–F) and spontaneous spiking has not been observed (De Biase et al., 2010). As the density of K^+ channels, and thereby the K_v/Na_v ratio, increases in adult animals, the ability of $NG2^+$ cells to generate spikes disappears (De Biase et al., 2010; Maldonado et al., 2011).

The molecular identity of the Na_v channels that support this form of excitability of $NG2^+$ cells has not been thoroughly investigated. One study analyzing the TTX-sensitivity of hippocampal $NG2^+$ cell Na^+ currents concluded that the IC_{50} of TTX (39.3 nM) of these currents was more compatible with an ensemble expression of multiple subunit types, rather than any individual subunit (Xie et al., 2007). This hypothesis is supported by the RNA-Seq database, which shows mRNA for $Na_v1.3$, $Na_v1.2$, $Na_v1.8$, and Nav1.1 (See Table 2). The beta subunits $Na_v\beta_1$, $Na_v\beta_3$, and $Na_v\beta_2$ are also expressed. So, it is likely that a heterogeneous mix of Na_v channel subunits contribute to the voltage dependent Na^+ currents.

2.2.3. Voltage-gated Ca^{2+} channels

Ca^{2+} signaling has been shown regulate many $NG2^+$ cell and oligodendrocyte behaviors, including proliferation, migration, process extension, differentiation, and myelination (Cheli et al., 2015; Paez et al., 2009; Simpson and Armstrong, 1999; Soliven, 2001; Yoo et al., 1999). There are several potential mechanisms of Ca^{2+} elevation in $NG2^+$ cells, including direct influx through plasma membrane voltage- or ligand-gated channels and release from internal Ca^{2+} stores. The presence of voltage-gated Ca^{2+} channels in $NG2^+$ cells is particularly interesting, as they could directly link depolarization from synaptic activity to Ca^{2+}-triggered changes in cell behavior.

Electrophysiological recordings from "complex glia" in a high-Ba^{2+}, low-Na^+ bath solution revealed voltage-dependent, divalent cation-mediated inward currents, indicative of Ca^{2+} channels (Akopian et al., 1996; Berger et al., 1992a). Later studies confirmed the presence of these currents in $NG2^+$ cells and found that they were sensitive to inhibitors of L-type and T-type Ca^{2+} channels (Fulton et al., 2010; Haberlandt et al., 2011). However, the currents were small, indicating a low overall density.

The molecular identity of voltage-gated Ca^{2+} channel subunits in $NG2^+$ cells has been examined in situ using single cell RT-PCR (Haberlandt et al., 2011). Consistent with pharmacological data, mRNA for L-type ($Ca_v1.2$ and $Ca_v1.3$), T-type ($Ca_v3.1$ and $Ca_v3.2$), P/Q-type ($Ca_v2.1$), and N-type ($Ca_v2.2$) α_1 subunits was detected. These findings are supported by the RNA-Seq transcriptome database, which yields a nearly identical list of highly-expressed α_1 subunits, along with a number of β, γ, and $\alpha_2\delta$ subunits (see Table 3). $Ca_v2.3$, an R-type subunit which was not detected by single-cell RT-PCR (Haberlandt et al., 2011) but was detected by RNA-Seq, is the only point of difference between the two datasets. $Ca_v1.2$ appears to be the principal pore-forming subunit, as siRNA knockdown of $Ca_v1.2$ in $NG2^+$ cells eliminates ~75% of the Ca^{2+} elevation following depolarization (Cheli et al., 2015).

Table 3 – Ca^{2+} channels.

Gene Name	Also known as	Expression level in NG2+ cells (FPKM)	Additional information (Catterall et al., 2005b)
Cacng4	TARPγ4	275.8	γ subunit[a]
Cacng7	TARPγ7	103.0	γ subunit[a]
Cacnb3	Cavβ3	25.7	β subunit
Cacng8	TARPγ8	12.0	γ subunit[a]
Cacng5	TARPγ5	11.7	γ subunit[a]
Cacnb4	Cavβ4	8.4	β subunit
Cacna2d3	Cavα2δ3	8.0	α2δ subunit
Cacna1d	Cav1.3	7.8	α1 subunit, L-type
Cacna1g	Cav3.1	7.7	α1 subunit, T-type
Cacnb1	Cavβ1	7.2	β subunit
Cacna2d1	Cavα2δ1	7.0	α2δ subunit
Cacna1c	Cav1.2	6.5	α1 subunit, L-type
Cacna1a	Cav2.1	6.5	α1 subunit, P/Q-type
Cacna1e	Cav2.3	6.0	α1 subunit, R-type
Cacna1h	Cav3.2	4.1	α1 subunit, T-type
Cacna2d2	Cavα2δ2	2.8	α2δ subunit
Cacna1b	Cav2.2	2.2	α1 subunit, N-type

[a] Associates with both Ca^{2+} channels and AMPARs (Black, 2003).

Table 4 – Cl^- channels.

Gene Name	Also known as	Expression level in NG2+ cells (FPKM)	Additional information (Walz, 2002)
Clcn2	Clc2	9.2	Hyperpolarization-activated Inward rectifying

2.2.4. Voltage-gated Cl⁻ channels

In addition to cation channels, NG2⁺ cells express voltage-gated Cl⁻ channels. The RNA-Seq transcriptome database reveals that Clc2 (Clcn2) is the only plasma membrane voltage-gated Cl⁻ channel gene expressed in NG2⁺ cells (See Table 4). This channel has inward-rectifying properties and a small unitary conductance (Walz, 2002), and can be activated by hyperpolarization, cell swelling, increased intracellular Cl⁻ concentration, or mild extracellular acidification (Jentsch, 2008).

Whole cell patch-clamp recordings from "complex" astrocytes in hippocampal and cortical brain slices have revealed a hyperpolarization-activated anion current that is absent in Clc2⁻/⁻ mice (Makara et al., 2003), supporting the expression of this channel by NG2⁺ cells. Immunohistochemistry and in situ hybridization have confirmed Clc2 expression in GFAP⁺ astrocytes and CC1⁺ oligodendrocytes, although NG2⁺ cells specifically have not been addressed with these techniques (Blanz et al., 2007). Clc2⁻/⁻ mice display extensive and progressive vacuolation of myelin, supporting an important role in oligodendroglial cell function (Blanz et al., 2007).

2.3. Other ion channels

2.3.1. Hyperpolarization-activated cyclic nucleotide-gated (HCN) channels

Hyperpolarization-activated cyclic nucleotide-gated (HCN) channels, part of the tetrameric voltage-gated K⁺ channel superfamily, are permeable to both Na⁺ and K⁺ (Wahl-Schott and Biel, 2009). They are activated by membrane hyperpolarization, giving rise to "anomalous rectification," and many are open at the resting membrane potential in both neurons and glia (Shah, 2014). The biophysical properties of these channels, as well as the extent of modulation by cyclic nucleotides, varies with subunit composition (Santoro et al., 2000). There are four subunit isoforms, HCN1-4, and both homomeric and heteromeric channels can be formed in vivo (Wahl-Schott and Biel, 2009). These channels are responsible for the

so-called I_h current in neurons, which is believed to contribute to maintenance of the RMP and to form part of the baseline membrane conductance, in addition to serving a pacemaking function in rhythmically-firing neurons (Santoro et al., 2000; Shah, 2014). Physiologically, NG2⁺ cells display evidence of anomalous rectification that is consistent with HCN channel expression, in which the membrane potential sags back to the resting potential upon hyperpolarization (Bergles et al., 2000).

The RNA-Seq transcriptome database reveals significant expression of two HCN channel subunits in NG2⁺ cells: HCN2 mRNA was present at higher levels than HCN3 (See Table 5). An early study using in situ hybridization in adult mice found HCN2 expression in a scattered population of cells in white matter tracts, suggesting its expression in glial cells in addition to neurons (Santoro et al., 2000). A later study in adult rat found that HCN2 antibody staining colocalized extensively with GST-π, a marker of mature oligodendrocytes, although not with NG2 (Notomi and Shigemoto, 2004). The transcriptome database shows that HCN2 mRNA expression in myelinating oligodendrocytes is four to five times higher than in NG2⁺ cells (Zhang et al., 2014); it is possible that the lower level of expression in NG2⁺ cells may not be sufficient for antibody detection. It is also possible that age differences between the studies could contribute to the discrepancy, as HCN2 protein levels are known to increase with age during postnatal development in the hippocampus (Surges et al., 2006).

2.3.2. TRP channels

The transient receptor potential (TRP) family of cation channels is very diverse, with a wide range of activation mechanisms, including ligand binding, G-protein coupled receptor (GPCR) activation, and physical stimuli such as temperature or pressure (Vennekens et al., 2012). The family can be divided into three subfamilies: the classic/canonical TRPs (TRPCs), the vanilloid receptor-related TRPs (TRPVs), and the melastatin-related TRPs (TRPMs). Several TRP channels are known to be expressed in glial cells: TRPC1 and TRPC3, nonselective cation channels activated by the phospholipase C signaling cascade, have been found in NG2⁺ cells and oligodendrocytes, respectively (Fusco et al., 2004; Paez et al., 2011; Weerth et al., 2007). TRPM3, a nonspecific cation channel activated by hypotonicity and sphingosine, has been found in both NG2⁺ cells and oligodendrocytes (Hoffmann et al., 2010).

According to the RNA-Seq transcriptome database, the most highly-expressed TRP channel subunit in NG2⁺ cells is TRPM7, a nonspecific cation-permeable channel that contains

Table 5 – HCN channels.

Gene Name	Also known as	Expression level in NG2⁺ cells (FPKM)	Additional Information (Wahl-Schott and Biel, 2009)
Hcn2	HAC1, BCNG2	14.8	Mixed Na⁺/K⁺ current
Hcn3	HAC3, BCNG4	3.7	Mixed Na⁺/K⁺ current

Table 6 – TRP channels.

Gene Name	Also known as	Expression level in NG2⁺ cells (FPKM)	Additional information (Clapham et al., 2005)
Trpm7	CHAK-1, LTrpC-7	9.2	Contains a Ser-Thr kinase domain. Outward rectifier. Permeable to Na⁺, Ca²⁺, and Mg²⁺
Trpc1	Trp1	4.2	Nonselective cation channel
Trpc2	mTrp2	2.6	Mouse pheromone channel, not expressed in humans
Trpm3	LTrpC-3	2.2	Nonselective cation channel

an ion pore fused to a serine-threonine kinase domain (Fleig and Chubanov, 2014) (see Table 6). This channel is widely expressed and is likely important for the importation and homeostasis of trace metal ions (Monteilh-Zoller et al., 2003). It has also been demonstrated to play a role in proliferation, migration, differentiation, and death of a variety of cell types (Fleig and Chubanov, 2014). However, the role of TRPM7 has not been specifically examined in NG2$^+$ cells. The TRP channels that have been previously linked to NG2$^+$ cells, TRPC1 and TRPM3, are found at low but significant levels in the transcriptome database (see Table 6), along with TRPC2, a gene that is expressed in rodents, but is a pseudogene in humans (Clapham et al., 2005).

3. Receptors

3.1. Glutamate receptors

3.1.1. Ionotropic glutamate receptors

The ionotropic glutamate receptor family consists of four subfamilies: α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors, kainate receptors, N-methyl-D-aspartate (NMDA) receptors, and δ receptors. δ receptors are a poorly-understood class of 'orphan' receptors which bind peptides such as Cbln1, a member of the C1q tumor necrosis factor superfamily (Matsuda and Yuzaki, 2012), and play important roles in synaptogenesis (Hirano, 2012; Yasumura et al., 2012). These receptors are not activated by glutamate (Lomeli et al., 1993) and do not appear to function as ligand-gated ion channels in situ. In each of the other subfamilies, a variety of subunits assemble into tetrameric channels that are permeable to Na$^+$, K$^+$, and in some cases Ca^{2+}, and currents through these receptors exhibit reversal potentials near 0 mV (Traynelis et al., 2010). AMPA receptor subunits are designated GluA1-GluA4, kainate receptor subunits are designated GluK1-GluK5, and NMDA receptor subunits are designated GluN1, GluN2A-GluN2D, and GluN3.

Studies of acutely isolated and cultured NG2$^+$ cells revealed that application of the AMPA/kainate receptor agonists glutamate, quisqualate, or kainate elicited inward currents that were blocked by the antagonist CNQX (Barres et al., 1990; Borges et al., 1994; Steinhäuser et al., 1992; Wyllie et al., 1991). Concurrent studies of "complex glia" in brain slices confirmed the presence of such currents in situ (Berger et al., 1992b; Jabs et al., 1994; Steinhäuser et al., 1994). Analysis of the desensitization kinetics of these receptors (Patneau et al., 1994) and use of specific AMPA receptor antagonists (Bergles et al., 2000; Kukley and Dietrich, 2009) demonstrated that NG2$^+$ cells in acute slices express both AMPA and kainate receptors. AMPA/kainate receptors are activated in situ at direct glutamatergic synapses formed with NG2$^+$ cells (Bergles et al., 2000; Jabs et al., 2005). At present, NG2$^+$ cells are the only glial cells that have been shown to form direct synapses with neurons, and in all cases, they are postsynaptic; NG2$^+$ cells lack axon-like projections and EM analysis has not detected presynaptic specializations in their processes (Bergles et al., 2000; Ziskin et al., 2007).

RT-PCR of mRNA isolated from cultured NG2$^+$ cells detected mRNA for all AMPA/kainate receptor subunits except GluK1, although GluA1 expression is very low (Holzwarth et al., 1994; Itoh et al., 2002; Patneau et al., 1994; Yoshioka et al., 1996). Single-cell RT-PCR from freshly isolated hippocampal "complex glia" revealed that GluA2, the subunit that renders AMPARs Ca^{2+}-impermeable in its RNA-edited form, was expressed by all cells in heterogeneous combinations with the other subunits (Seifert et al., 1997a). Universal expression of GluA2 in cultured NG2$^+$ cells has also been shown by immunocytochemistry (Deng et al., 2006). Moreover, >95% of the GluA2 mRNA in cultured NG2$^+$ cells is edited to the Ca^{2+}-impermeable form (Itoh et al., 2000; Puchalski et al., 1994). Together, these data suggest that NG2$^+$ cells express only Ca^{2+}-impermeable AMPARs. However, through analysis of the current-voltage relationship of agonist-induced responses, the use of antagonists specific to GluA2-containing AMPARs, and direct Ca^{2+} imaging of NG2$^+$ cells in response to glutamate, it has been repeatedly shown that NG2$^+$ cells express functional Ca^{2+}-permeable AMPARs (Bergles et al., 2000; Ge et al., 2006b; Holzwarth et al., 1994; Itoh et al., 2002; Seifert et al., 1997b). Thus, it is likely that individual NG2$^+$ cells express both Ca^{2+}-permeable and impermeable AMPARs within the same cell, a hypothesis supported by biochemical evidence (Deng et al., 2006; Itoh et al., 2002). The relative proportions of these receptors vary over time and even cellular domain, as Ca^{2+}-permeable AMPARs are more highly expressed in younger animals and at extrasynaptic sites (Ge et al., 2006b), and their abundance can be affected by plasticity (Ge et al., 2006b; Zonouzi et al., 2011).

The RNA-Seq transcriptome database is in good agreement with the findings of these previous studies (see Table 7). GluA2 is by far the most highly-expressed AMPA/kainate receptor subunit, followed by GluA3, GluK5, and GluA4. The remaining kainate receptor subunits and the AMPA receptor subunit GluA1 are expressed at lower, but still significant levels.

The function of AMPA/kainate receptors in NG2$^+$ cells in vivo is not understood, but evidence from in vitro studies indicates that they likely regulate numerous NG2$^+$ cell behaviors. AMPA/kainate receptor antagonists have been shown to stimulate NG2$^+$ cell migration in vitro (Gudz et al., 2006), and application of agonists to NG2$^+$ cells in dissociated culture or cultured brain slices has been shown to inhibit their proliferation and differentiation, likely by regulating the activity of K$^+$ channels (Gallo et al., 1996; Yuan et al., 1998). Silencing of neuronal activity (and thereby glutamate release) with TTX or blockade of AMPARs was recently shown to increase NG2$^+$ cell proliferation and differentiation in cerebellar slice cultures (Fannon et al., 2015). Notably, however, this increase in differentiation was not accompanied by an increase in myelination. Deciphering the involvement of this form of signaling in regulating NG2$^+$ cell behavior in vivo will require direct manipulation of AMPA/kainate receptor expression within these cells, to exclude possible indirect effects on surrounding neurons.

The majority of initial studies of NG2$^+$ cells in culture (Barres et al., 1990; Holzwarth et al., 1994; Patneau et al., 1994; Wyllie et al., 1991) and in acute brain slices (Berger et al., 1992b) found no evidence of NMDA receptor-mediated currents. However, one early study of cultured NG2$^+$ cells

Table 7 – Glutamate receptors.

Gene Name	Also known as	Expression level in NG2$^+$ cells (FPKM)	Additional information (Traynelis et al., 2010)
Gria2	GluA2, GluR2	108.4	AMPAR
Gria3	GluA3, GluR3	54.4	AMPAR
Grik5	GluK5, KA2	47.4	Kainate
Gria4	GluA4, GluR4	37.8	AMPAR
Grid1	GluD1, δ1	30.1	Orphan
Grin3a	GluN3A	23.5	NMDAR
Grik4	GluK4, KA1	19.3	Kainate
Grin1	GluN1, NR1	16.3	NMDAR
Grik3	GluK3, GluR7	14.9	Kainate
Grik2	GluK2, GluR6	14.0	Kainate
Grm5	mGluR5	13.3	Metabotropic (Group I)
Grik1	GluK1, GluR5	12.7	Kainate
Grid2	GluD2, δ2	11.9	Orphan
Gria1	GluA1, GluR1	10.4	AMPAR
Grm3	mGluR3	7.7	Metabotropic (Group II)
Grin2c	GluN2C, NR2C	5.5	NMDAR
Grin2d	GluN2D, NR2D	3.9	NMDAR
Grm4	mGluR4	2.1	Metabotropic (Group III)

reported that small inward currents were elicited in response to NMDA that were more prominent at depolarized potentials, were sensitive to extracellular Mg^{2+}, and were inhibited by the NMDAR antagonist MK-801 (Wang et al., 1996). Intracellular Ca^{2+} elevations, sensitive to the antagonist AP5, were also observed in response to NMDA application (Wang et al., 1996). mRNA for the GluN1 subunit has also been detected by RT-PCR from cultured NG2$^+$ cells (Wang et al., 1996; Yoshioka et al., 1996). Subsequent studies have extended these findings in brain slices and isolated optic nerves, showing with pharmacology, RT-PCR, and immunohistochemistry that functional NMDARs are expressed throughout the oligodendrocyte lineage (De Biase et al., 2010; Káradóttir et al., 2005; Micu et al., 2006; Salter and Fern, 2005; Ziskin et al., 2007). However, electrophysiological recordings have shown that NMDAR expression in NG2$^+$ cells is not universal (only ~60% of cells in corpus callosum responded to NMDA), and the density of the receptors appears to be very low, with a maximal excitatory postsynaptic current (EPSC) of ~10 pA at 30 mV (Ziskin et al., 2007).

The discrepancy between early studies in dissociated or cultured cells and later studies *in situ* suggests either that cultured NG2$^+$ cells downregulate their expression of NMDARs, or that NMDAR expression is disrupted by the papain used to isolate cells, as was found to be the case for retinal ganglion cells in similar conditions (Barres et al., 1990).

The RNA-Seq transcriptome database provides additional evidence of the expression of NMDARs by NG2$^+$ cells (See Table 7). The most highly-expressed subunits are GluN3A, GluN1, and, at a lower level, GluN2C and GluN2D. Receptors containing GluN3A, GluN2C, and GluN2D are known to have reduced sensitivity to extracellular Mg^{2+}, (Kuner and Schoepfer, 1996; Sasaki et al., 2002) and consistent with this observation, NMDAR-mediated currents in NG2$^+$ cells have been shown to be less sensitive to Mg^{2+} than those in neurons (Káradóttir et al., 2005) (but see (Ziskin et al., 2007)).

Although mature oligodendrocytes express GluN1, GluN2C, and GluN3A subunits by immunohistochemistry and RT-PCR,

(Káradóttir et al., 2005; Salter and Fern, 2005) physiological recordings in acute brain slices have shown that the density of NMDAR receptor expression is markedly reduced as NG2$^+$ cells transition to the pre-myelinating stage (De Biase et al., 2010). The RNA-Seq database corroborates that expression of NMDARs declines upon NG2$^+$ cell differentiation. For example, GluN1 mRNA expression is ~five-fold lower in newly formed oligodendrocytes than in progenitors (Zhang et al., 2014).

The function of NMDARs in NG2$^+$ cells *in vivo* is not clear. NMDARs have been demonstrated to regulate the development and survival of neural progenitors (Ikonomidou et al., 1999; Jansson and Akerman, 2014; Nacher and McEwen, 2006). However, it has been shown that they are not required for NG2$^+$ cell survival, formation of synaptic connections with neurons, or differentiation into oligodendrocytes *in vivo*, but may be important in regulating the expression of Ca^{2+}-permeable AMPA receptors (De Biase et al., 2011). Recent studies indicate that, in the presence of neuregulin (NRG) or BDNF, glutamate signaling through NMDA receptors in oligodendroglia promotes activity-dependent myelination of axons in a co-culture system (Lundgaard et al., 2013). Addition of NRG or BDNF to culture media decreased the expression of the NR3A subunit and significantly increased the amplitude of NMDAR-mediated currents in NG2$^+$ cells and oligodendrocytes. Addition of these factors also rendered myelination dependent on neuronal action potential firing and glutamate release. Interestingly, it was shown that remyelination after white matter damage *in vivo* is inhibited by NMDAR blockade, supporting the *in vivo* relevance of this NMDAR-dependent myelination program (Lundgaard et al., 2013). The relative contributions of NG2$^+$ cells and mature oligodendrocytes to this phenomenon need to be elucidated, but these results point to an important role of NMDARs in regulating myelination.

3.1.2. Metabotropic glutamate receptors

Studies using RT-PCR, immunohistochemistry, and Western blot have shown that cultured NG2$^+$ cells express group I (mGluR1 and mGluR5), group II (mGluR3) and group III (mGluR4) metabotropic glutamate receptors (Deng et al.,

2004; Luyt et al., 2006, 2003; Spampinato et al., 2015a). Functional studies have focused mainly on group I mGluRs, which elevate intracellular Ca^{2+} by activating phospholipase C (Niswender and Conn, 2010). In brain slices, the specific group I mGluR agonist 3,5-DHPG induces Ca^{2+} increases without accompanying current responses in hippocampal $NG2^+$ cells, and this response is blocked by preincubation with the mGluR antagonist LY341495 (Haberlandt et al., 2011). The use of subunit-specific antagonists 3-MATILDA and MPEP revealed that the expression of mGluR1 and mGluR5 varies among individual $NG2^+$ cells. Similar results had been previously observed in cultured $NG2^+$ cells (Luyt et al., 2003).

The RNA-Seq transcriptome database is largely concordant with these findings (See Table 7). The most highly-expressed mGluR subunits are mGluR5, mGluR3, and mGluR4, while mGluR1 is expressed only at very low levels (0.5 FPKM).

Activation of group I mGluRs and subsequent elevation of intracellular Ca^{2+} in $NG2^+$ cells has been implicated in promoting cell survival (Deng et al., 2004; Luyt et al., 2006) and in inducing plasticity of neuron-$NG2^+$ cell synapses (Zonouzi et al., 2011). In particular, treatment with 3,5-DHPG leads to an increase in the single-channel conductance of AMPARs and the proportion of calcium-permeable AMPARs in cultured $NG2^+$ cells. Such a mechanism may underlie the LTP-like synaptic potentiation that is observed at neuron-$NG2^+$ cell synapses in the hippocampus (W. P. Ge et al., 2006b). Additionally, activation of mGluR4, a group III mGluR, with the agonist L-AP4 was recently shown to accelerate the differentiation of cultured $NG2^+$ cells (Spampinato et al., 2015b). Although *in vivo* studies to confirm these findings have not been performed, these results point to an important role for mGluRs in regulating $NG2^+$ cell development and synaptic plasticity.

3.2. GABA receptors

3.2.1. Ionotropic GABA receptors

Cultured $NG2^+$ cells have been shown to express $GABA_A$ receptors, Cl^--permeable ion channels that are activated by the specific agonist muscimol and inhibited by biculculline (Von Blankenfeld et al., 1991; Williamson et al., 1998). These receptors were subsequently found in "complex glia" and in physiologically-identified $NG2^+$ cells in the hippocampus, cortex, and corpus callosum (Berger et al., 1992b; Lin and Bergles, 2004; Steinhäuser et al., 1994). GABA receptors in $NG2^+$ cells can be activated *in situ* by direct synaptic connections from inhibitory interneurons or by spillover of GABA from nearby synapses (Kukley et al., 2008; Lin and Bergles, 2004; Vélez-Fort et al., 2010).

It has been consistently observed that application of $GABA_A R$ agonists to $NG2^+$ cells results in depolarization and elevation of intracellular Ca^{2+} (Kirchhoff and Kettenmann, 1992; Lin and Bergles, 2004; Pastor et al., 1995; Tanaka et al., 2009; Tong et al., 2009; Von Blankenfeld et al., 1991). This is in contrast to mature neurons, in which the opening of Cl^- channels typically causes membrane hyperpolarization. Experiments performed in the perforated patch configuration, which preserves the native internal Cl^- concentration, revealed that $GABA_A$-mediated currents in $NG2^+$ cells have a reversal potential in the range of −30 to −40 mV,

significantly more positive than the RMP or the reversal potential of neuronal $GABA_A Rs$ ($\sim$ −70 mV) (Lin and Bergles, 2004; Passlick et al., 2013; Tanaka et al., 2009; Tong et al., 2009). These findings led to the conclusion that $NG2^+$ cells maintain higher internal Cl^- concentration than mature neurons. A similar phenomenon has been observed in other 'immature' proliferative cell populations in the neurogenic niches of the adult brain (Ge et al., 2006a; Wang et al., 2003).

$GABA_A$ receptors are heteropentamers typically composed of two α, two β, and one γ subunit (Olsen and Sieghart, 2008). Subunit composition affects the sensitivity, kinetics, pharmacological profile, and cell surface distribution of the receptor complex. The α subunit is the primary determinant of ligand sensitivity, with α6 showing the highest and α3 showing the lowest affinity (Farrant and Nusser, 2005; Gingrich et al., 1995). The α subunit is also an important determinant of response kinetics, with α1 associated with rapid decay time and α5 conferring a slow decay time (Burgard et al., 1996; Goldstein et al., 2002). The γ subunit, particularly γ2, is important for receptor clustering and synaptic localization (Farrant and Nusser, 2005).

RT-PCR showed that mixed glial cultures containing 85% $NG2^+$ cells contained mRNA for α2, α3, α4, α5, γ2, γ3, and low levels of γ1 (β sununits were not assessed) (Williamson et al., 1998). α3 is the most highly expressed subunit in culture, and α6 is absent, which is consistent with physiological data in culture and brain slices showing that $NG2^+$ cells have low-affinity, slowly-activating receptors (Jabs et al., 2005; Lin and Bergles, 2004; Williamson et al., 1998). $NG2^+$ cell GABAergic miniature EPSCs (mEPSCs) also have considerably slower decay kinetics than those of CA1 pyramidal neurons, consistent with expression of α5 and absence of α1 (Lin and Bergles, 2004).

Single-cell RT-PCR from genetically- and physiologically-identified $NG2^+$ cells in hippocampus of P9-12 mice and cortex of P7-11 and P21-29 mice showed considerable heterogeneity of $GABA_A R$ subunit expression (Balia et al., 2015; Passlick et al., 2013). Consistent with the *in vitro* studies, all α subunits except α6 were expressed to some extent; α3 and α4 were upregulated with age and α5 was downregulated. β2 and β3 were high at all ages while β1 was low. γ1 and γ3 were high at both time points, while γ2 subunit was dramatically downregulated with age. These findings were corroborated by physiological experiments using subunit-specific agonists and antagonists (Balia et al., 2015; Passlick et al., 2013). The finding that γ2 subunit expression, which is associated with synaptic localization, decreases with age correlates with a decrease in the frequency of GABAergic synaptic contacts on cortical $NG2^+$ cells as the animal matures (Balia et al., 2015; Vélez-Fort et al., 2010).

The RNA-Seq transcriptome database drawn from P17 animals lies in between the two time points measured by single cell RT-PCR (See Table 8). The concordance between the data sets is mixed: Among α subunits, α3 is the most highly expressed, while α2, α4, and α1 are expressed at lower levels. Surprisingly, α5 is expressed only at extremely low levels (0.6 FPKM). Among β subunits, in agreement with previous results, β3 and β2 are highly expressed while β1 is expressed at lower levels. Among γ subunits, γ1 is the most highly expressed while γ2 and γ3 are at low levels. It must be kept in mind that the transcription

Table 8 – GABA receptors.

Gene Name	Also known as	Expression level in NG2$^+$ cells (FPKM)	Additional information (Benarroch, 2012; Olsen and Sieghart, 2008)
Gabbr1	GABA$_B$R1	55.4	Metabotropic
Gabra3	GABA$_A$Rα3	23.7	Ionotropic
Gabrb3	GABA$_A$Rβ3	21.7	Ionotropic
Gabbr2	GABA$_B$R2	12.7	Metabotropic
Gabrg1	GABA$_A$Rγ1	12.2	Ionotropic
Gabrb2	GABA$_A$Rβ2	10.9	Ionotropic
Gabra2	GABA$_A$Rα2	5.8	Ionotropic
Gabra4	GABA$_A$Rα4	3.9	Ionotropic
Gabrg2	GABA$_A$Rγ2	3.7	Ionotropic
Gabra1	GABA$_A$Rα1	3.6	Ionotropic
Gabrb1	GABA$_A$Rβ1	3.5	Ionotropic
Gabrg3	GABA$_A$Rγ3	3.2	Ionotropic

database is derived from dissociated cells, which no longer are in synaptic contact with neurons, which may alter their expression of receptor subunits. As GABA$_A$ receptor activation induces depolarization, these receptors may work in concert with AMPA receptors to force greater membrane potential fluctuations and recruitment of other voltage-gated channels (e.g. Ca^{2+} channels).

3.2.2. Metabotropic GABA receptors

GABA$_B$ receptors are metabotropic G-protein coupled receptors that typically hyperpolarize cells by opening K$^+$ channels and inhibiting Ca$_v$ channels, while also inhibiting cAMP production. They are formed by heterodimerization of two 7-transmembrane subunits, GABA$_B$R1 and GABA$_B$R2 (Benarroch, 2012). Despite extensive characterization of GABA$_A$R expression and GABAergic synaptic innervation of NG2$^+$ cells, the question of whether GABA$_B$ receptors are also expressed by these cells was not addressed until relatively recently. Immunohistochemical analysis of NG2$^+$ cells in hippocampal slices, in addition to RT-PCR and Western blot in the NG2$^+$ cell-like CG-4 cell line, demonstrated that these cells express GABA$_B$R1 and GABA$_B$R2 (Luyt et al., 2007). Functional expression of these receptors has not been demonstrated electrophysiologically, but it was shown that the specific GABA$_B$R agonist baclofen causes a reduction in cAMP levels and promotes the proliferation and migration of CG-4 cells, indicating that functional receptors are likely expressed (Luyt et al., 2007). These findings have not yet been extended to NG2$^+$ cells in situ. In agreement with the findings from CG-4 cells, the RNA-Seq transcriptome database shows that GABA$_B$R1 and GABA$_B$R2 are among the most highly expressed GABAR subunits in NG2$^+$ cells (see Table 8).

3.3. Receptors for neuromodulators

3.3.1. Adrenergic receptors

Adrenergic receptors (ARs) are G protein-coupled receptors that respond to norepinephrine (NE) and epinephrine and are divided into three broad classes: α$_1$ receptors, which signal through G$_q$ and activate phospholipase C, α$_2$ receptors, which signal through G$_i$ and inhibit cAMP production, and β$_1$–β$_3$

receptors, which all signal through G$_s$ and stimulate cAMP production (Bylund et al., 1994). These classes have diverse expression profiles, ligand affinities, and pharmacology, and each can be further divided into multiple isoforms.

The presence of α$_{1A}$ receptors in cultured NG2$^+$ cells was first inferred based on a measured increase in IP$_3$ production in response to application of NE or the α$_1$-specific agonist phenylephrine. This response was blocked by a specific α$_{1A}$ antagonist, WB-4101, and not by antagonists to other subtypes (Cohen and Almazan, 1993). In a separate study, application of phenylephrine elevated intracellular Ca^{2+} levels in cultured NG2$^+$ cells (Kastritsis and McCarthy, 1993). When cultured NG2$^+$ cells were assayed using RT-PCR and Western blot, they were found to express mRNA and protein for α$_{1A}$, α$_{1B}$, and α$_{1D}$ receptor subtypes, while the use of specific antagonists pointed to α$_{1A}$ as the key player promoting IP$_3$ production in response to norepinephrine (Khorchid et al., 2002). The development of transgenic mice expressing EGP under the control of the α$_{1A}$ promoter, and another line overexpressing an α$_{1B}$-EGFP fusion protein, allowed for the localization of these proteins in the adult brain, and confirmed that both subtypes are expressed by NG2$^+$ cells (Papay et al., 2006, 2004).

Application of the specific β-AR agonist isoproterenol to cultured NG2$^+$ cells causes a significant accumulation of cAMP, indicating that functional β-ARs are also expressed (Ghiani et al., 1999). Activation of β-ARs with NE or isoproterenol, either in dissociated NG2$^+$ cell cultures or in organotypic cerebellar slice cultures, leads to G$_1$ cell cycle arrest, inhibition of cell proliferation, and increased differentiation (Ghiani et al., 1999). However, an early study of cultured glial cells found no immunostaining for β-ARs on NG2$^+$ cells, despite finding expression on astrocytes and oligodendrocytes (Ventimiglia et al., 1987). To our knowledge, no studies have assayed β-AR expression in NG2$^+$ cells using RT-PCR.

The RNA-Seq transcriptome database shows only very low expression levels of adrenergic receptors by NG2$^+$ cells (see Table 9); however, fewer receptors may be required, due to their high degree of intracellular signal amplification. β$_2$ and α$_{1A}$ were the only subunits to surpass the 2 FPKM threshold, though β$_2$, which is expressed highly in microglia (~100 FPKM), may be influenced by microglial contamination. β$_1$, α$_{1B}$, and α$_{2A}$ were expressed below the threshold (1.5, 1.4, and 1.4 FPKM, respectively). It may be that mRNA and protein levels are discordant, that the receptors are upregulated in culture, or that such low expression levels are still sufficient to mediate the observed responses. The functional significance of adrenergic signaling through these different receptor classes in vivo remains unexplored.

3.3.2. Acetylcholine receptors

Nicotinic acetylcholine receptors (nAChRs) are ionotropic receptors that pass Na$^+$, K$^+$, and sometimes Ca^{2+}, and are activated by nicotine. They are pentamers composed of 16 possible subunits: α$_1$-α$_9$, β$_1$-β$_4$, γ, δ, and ε. Only certain combinations are found in vivo, and the most common combinations in the CNS are α$_4$β$_2$, α$_4$α$_5$β$_2$, and α$_7$ homomers (Lukas et al., 1999).

Cultured cortical NG2$^+$ cells exhibited inward currents in response to acetylcholine that were blocked by the nicotinic

Table 9 – Receptors for neuromodulators.			
Gene Name	Also known as	Expression level in NG2$^+$ cells (FPKM)	Additional information ([Abbracchio et al., 2006]; [Burnstock et al., 2011]; [Bylund et al., 1994]; [Caulfield and Birdsall, 1998]; [Fredholm et al., 2011]; [Khakh et al., 2001]; [Lukas et al., 1999]; [Lynch, 2009])
P2ry12	P2Y$_{12}$	67.0[a]	Metabotropic purinergic receptor
Adora1	A$_1$	41.9	Adenosine receptor
Chrna4	nAChRα4	37.7	Nicotinic acetylcholine receptor
Glrb	GlyRβ	36.8	Glycine receptor
P2rx7	P2×$_7$	21.0	Ionotropic purinergic receptor
Adora2b	A$_{2B}$	8.8	Adenosine receptor
P2rx4	P2×$_4$	5.6	Ionotropic purinergic receptor
P2ry1	P2Y$_1$	5.0	Metabotropic purinergic receptor
P2ry6	P2Y$_6$	2.7[a]	Metabotropic purinergic receptor
Adrb2	β$_2$AR	2.4[a]	β adrenergic receptor
Chrna7	nAChRα7	2.2	Nicotinic acetylcholine receptor
Chrm1	mAChR1	2.1	Muscarinic acetylcholine receptor
Adra1A	α$_{1A}$AR	2.1	α adrenergic receptor
Chrnb2	nAChRβ2	2.0	Nicotinic acetylcholine receptor
P2ry2	P2Y$_2$	2.0	Metabotropic purinergic receptor

[a] These genes have very high expression in microglia, which are known to be a 5% contaminant in the NG2$^+$ cell sample.

antagonist hexamethonium, ([Belachew et al., 1998a]) and RT-PCR and immunocytochemistry demonstrated that cultured NG2$^+$ cells derived from corpus callosum expressed α$_4$ and β$_2$ nAChRs at high levels, and α$_3$, α$_5$, α$_7$, and β$_4$ at lower levels ([Rogers et al., 2001]). Application of nicotine to the callosal cultures led to Ca^{2+} oscillations in NG2$^+$ cells. In acute hippocampal slices of juvenile mice, recordings from electrophysiologically-identified NG2$^+$ cells revealed that they express the highly Ca^{2+}-permeable α$_7$ homomeric receptor, which is potentiated by PNU-120596 and inhibited by methyllycaconitine. In contrast to the RT-PCR data from culture, no evidence of DHβE-sensitive α$_4$β$_2$ receptors was observed in these cells ([Shen and Yakel, 2012]; [Vélez-Fort et al., 2009]).

The RNA-Seq transcriptome database shows that the most highly-expressed nAChR subunit is α$_4$, distantly followed by α$_7$ and β$_2$ (See Table 9). This list is shorter than, but consistent with, the list of subunits identified in cultured NG2$^+$ cells by RT-PCR. It points to the expression of α$_7$ homomers or α$_4$β$_2$ heteromers, although the expression level of α$_4$ is 17-fold higher than β$_2$. The differences between the RT-PCR/RNA-Seq data and the pharmacological data could be due to differences between mRNA and protein expression, or regional differences between hippocampus and cortex. Pharmacological studies of cortical NG2$^+$ cells in brain slices would help resolve this issue.

Muscarinic acetylcholine receptors (mAChRs) are metabotropic G protein-coupled receptors. There are five subtypes, designated M$_1$–M$_5$. M$_1$, M$_3$, and M$_5$ signal through the G$_q$ protein, resulting in intracellular Ca^{2+} elevation, while M$_2$ and M$_4$ signal through the G$_{i/o}$ protein, resulting in inhibition of cAMP production. To date, the only studies analyzing mAChR expression in NG2$^+$ cells have been performed in culture, mainly by measuring cellular changes in response to the acetylcholine analog carbachol. A series of studies demonstrated that carbachol induces IP$_3$ generation, inhibits cAMP accumulation, causes an elevation of intracellular Ca^{2+}, induces c-fos expression, and ultimately promotes cell survival and proliferation ([Cohen and Almazan, 1994]; [Cohen et al.,

1996]; [Cui et al., 2006]; [Kastritsis and McCarthy, 1993]; [Larocca and Almazan, 1997]). (However, one study found no effect on proliferation, see ([Gallo et al., 1996])). All of these effects were blocked by atropine, a non-specific mAChR antagonist. RT-PCR analysis has provided further evidence of M$_1$ and M$_2$ receptor expression ([Cohen and Almazan, 1994]). In a follow-up study, more extensive RT-PCR analysis by the same group showed that M$_3$ is the most highly expressed isoform, followed by M$_4$, and M$_1$, M$_2$, and M$_5$ at lower levels ([Ragheb et al., 2001]). It was also demonstrated that the aforementioned effects on NG2$^+$ cells are mainly mediated by M$_3$ receptors, as they were inhibited by the selective M$_3$ antagonist 4-DAMP ([Ragheb et al., 2001]).

Similar to that observed for adrenergic receptors, the RNA-Seq transcriptome database shows only very low levels of mAChR mRNA expression by NG2$^+$ cells (See Table 9). Only one isoform, M$_1$, passed the threshold, at 2.1 FPKM. M$_2$-M$_5$ fell within the 0.1-1.9 FPKM range. Thus far, all studies of oligodendroglial mAChR expression have been performed on cultured cells, so it is possible that high mAChR expression is induced by the in vitro environment. Although mAChRs have been pharmacologically identified in astrocytes in acute hippocampal slices ([Shelton and McCarthy, 2000]), similar studies need be performed on NG2$^+$ cells to determine if the expression pattern holds in situ.

Recent evidence for the functional importance of mAChRs in NG2$^+$ cells comes from several high-throughput screening studies aimed at identifying therapeutic targets in demyelinating disorders. Benztropine, an antagonist of M$_1$ and M$_3$ receptors, strongly promoted expression of myelin basic protein (MBP) by differentiating NG2$^+$ cells, and was effective at enhancing remyelination and reducing clinical severity in several animal models of multiple sclerosis ([Deshmukh et al., 2013]). A cluster of antimuscarinic compounds was also identified as promoting NG2$^+$ cell differentiation in a study that assessed the formation of myelin-like wraps around small conical micropillars, and the muscarinic antagonist clemastine was shown to promote oligodendrocyte

differentiation and remyelination after demyelination *in vivo* (Mei et al., 2014). Finally, of particular relevance to humans, M_3 expression was identified in a microarray analysis of FACS-sorted fetal human forebrain OPCs (Abiraman et al., 2015). Treatment of cultured human OPCs with the muscarinic agonist oxotremorine-M prevented their differentiation to oligodendrocytes, while the specific M_3 antagonist darifenacin promoted differentiation. Moreover, treatment with the M_3 antagonist solifenacin increased differentiation of engrafted human OPCs in a demyelinated mouse model. These data provide additional evidence that muscarinic acetylcholine receptors play important roles in $NG2^+$ cell biology, and are exciting targets for pharmacotherapy in demyelinating disorders.

3.3.3. Dopamine receptors

The dopamine receptor family consists of five G protein-coupled receptors that can be divided into two classes: D1 class (D1 and D5 receptors), which signal through G_s and stimulate cAMP production, and D2 class (D2, D3, and D4 receptors), which signal through $G_{i/o}$ and inhibit cAMP production (Beaulieu and Gainetdinov, 2011).

D3 receptors, but not D2 receptors, were found by RT-PCR and Western blot in primary mixed glial cultures from newborn mice, while *in situ* hybridization and immunocytochemistry showed that the expression was restricted to $NG2^+$ cells in the cultures (Bongarzone et al., 1998). In the same study D3 receptors were also found by immunohistochemistry in oligodendrocyte-like cells in the corpus callosum of juvenile, but not adult, mice. Stimulation of D3 receptors in $NG2^+$ cells *in vitro* with quinpirole increases their differentiation (Bongarzone et al., 1998). A subsequent study confirmed D3 receptor mRNA expression in cultured $NG2^+$ cells and showed that haloperidol, a D2/D3 inhibitor, promotes their proliferation and inhibits their differentiation (Niu et al., 2010).

Somewhat surprisingly given these results, the RNA-Seq database shows no significant expression of any dopamine receptors in $NG2^+$ cells (Zhang et al., 2014). It is possible that the receptor expression previously observed may be unique to the culture environment, as there is limited evidence for their expression by $NG2^+$ cells *in situ*. It is also possible that dopamine receptor expression *in vivo* is enriched in brain regions where dopamine is generated and released, such as the ventral tegmental area and nucleus accumbens, and is therefore not represented in available datasets from the hippocampus or cortex. If dopamine receptors are expressed by $NG2^+$ cells *in vivo*, oligodendrogenesis and myelination within reward pathways may be affected by drugs of abuse that act by modulating the dopamine system (see Discussion).

3.3.4. Glycine receptors

Glycine receptors (GlyRs) are ionotropic receptors composed of five homo- or herteromerically-assembled subunits. GlyRs conduct Cl^- and are known to be mediators of synaptic inhibition in the brainstem and spinal cord (Lynch, 2009). Four α ($α_1$-$α_4$) and one β subunit have been identified. Unlike α subunits, the β subunit alone cannot form homomeric channels (Griffon et al., 1999) or produce glycine-gated currents (Bormann and Rundstrom, 1993) in heterologous systems. Application of glycine has been shown to induce an inward current and Ca^{2+} elevation in cultured cortical $NG2^+$ cells, and these responses were inhibited by the GlyR antagonist strychnine and the Cl^- channel blocker picrotoxin (Belachew et al., 2000, 1998a, 1998b). Similar currents have been observed in unidentified glial precursor cells in rat spinal cord slices (Kirchhoff et al., 1996; Pastor et al., 1995).

RT-PCR and immunocytochemistry from cortical $NG2^+$ cell cultures show expression of the $α_2$ and β subunits but not $α_1$ and $α_3$ (Belachew et al., 1998b). However, single cell RT-PCR of glial precursor cells from rat spinal cord slices found expression of the $α_1$ and β subunits, but not $α_2$ or $α_3$ (Kirchhoff et al., 1996). In contrast to both of these studies, the RNA-Seq database shows expression of only the GlyR β subunit, with no expression of any α subunits (see Table 9), a scenario that would not result in formation of functional receptors. Since glycinergic signaling is much more prominent in the spinal cord, it is possible that $NG2^+$ cells in that region have much higher expression of GlyRs to enable these progenitors to be influenced by neuronal activity.

3.3.5. Purinergic receptors

Purinergic receptors are divided into two broad classes: P1 receptors, which are activated by adenosine, and P2 receptors, which are activated by ATP or other nucleotides.

P1 receptors are G protein-coupled receptors, of which there are four vertebrate homologs: A_1, A_{2A}, A_{2B}, and A_3. A_1 and A_3 are coupled to $G_{i/o}$, while A_{2A} and A_{2B} are coupled to G_s (Fredholm et al., 2001). By RT-PCR, cultured and freshly isolated cortical $NG2^+$ cells have been shown to express all four adenosine receptors (Stevens et al., 2002), and expression of A_1 has been shown by immunocytochemistry (Othman et al., 2003). Application of specific adenosine receptor agonists to cultured $NG2^+$ cells has been observed to increase their migration (Othman et al., 2003) and increase (Stevens et al., 2002) or decrease (Coppi et al., 2013) their differentiation, depending on the receptor subtype activated.

A_1 is the most highly expressed adenosine receptor in $NG2^+$ cells in the RNA-Seq database, followed by A_{2B} (see Table 9). A_{2A} and A_3 are expressed only at very low levels (0.9–1.7 FPKM). Prior RT-PCR experiments were performed from $NG2^+$ cells isolated from mice at P1, so the observed differences in expression may reflect changes in gene expression patterns with development. Further functional studies are needed to determine the impact of these distinct receptors on $NG2^+$ cell behavior.

P2 receptors can be divided into two broad classes: P2X and P2Y. P2X receptors are ligand-gated ion channels. There are seven P2X receptor subunits, $P2X_1$-$P2X_7$, and they form oligomeric nonselective cation channels permeable to Na^+, K^+, and Ca^{2+} (Dal Ben et al., 2015; Khakh et al., 2001). Both homomeric and heteromeric subunit combinations are possible. P2Y receptors are G protein-coupled receptors, of which there are eight mammalian subtypes: $P2Y_1$, $P2Y_2$, $P2Y_4$, $P2Y_6$, $P2Y_{11}$, $P2Y_{12}$, $P2Y_{13}$, and $P2Y_{14}$. These vary in their expression pattern, ligand specificity, and second-messenger signaling. $P2Y_1$, $P2Y_2$, $P2Y_4$, $P2Y_6$, and $P2Y_{11}$ couple to G_q, while $P2Y_{12}$, $P2Y_{13}$, and $P2Y_{14}$ couple to $G_{i/o}$ (Abbracchio et al., 2006; Weisman et al., 2012).

NG2$^+$ cells in culture and in brain slices respond to ATP with intracellular Ca^{2+} elevations (Agresti et al., 2005a; Bernstein et al., 1996; Hamilton et al., 2010; He and McCarthy, 1994). These elevations are not affected by changes in the external Ca^{2+} concentration but sensitive to depletion of internal Ca^{2+} stores, supporting a role of the metabotropic P2Y receptors (Kirischuk et al., 1995). It should be noted that some studies did not detect Ca^{2+} elevations in the most immature cultured oligodendrocyte precursors (Kastritsis and McCarthy, 1993) or in the majority of presumed oligodendrocyte precursors from early postnatal corpus callosum slices (Kirischuk et al., 1995). But, using the NG2-DsRed line to unambiguously identify NG2$^+$ cells in the juvenile mouse corpus callosum, it was demonstrated that these cells respond to exogenous ATP with increases in intracellular Ca^{2+} (Hamilton et al., 2010) It was also shown that nearby astrocytes are a potential source of ATP, which release ATP response to action potential firing by axons (Hamilton et al., 2010). Use of specific agonists and antagonists showed that P2Y$_1$ and P2$\times_7$ receptors are major contributors to this NG2$^+$ cell response.

Using Western blot in NG2$^+$ cell cultures, expression of P2$\times_1$-P2$\times_4$, P2$\times_7$, P2Y$_1$, P2Y$_2$, and P2Y$_4$ receptors has been demonstrated (Agresti et al., 2005a, 2005b). Protein expression of P2Y$_1$ by NG2$^+$ cells in situ has also been demonstrated by immunohistochemistry (Agresti et al., 2005a). Use of specific agonists and antagonists has confirmed that P2$\times_7$ and P2Y$_1$ are the major purinergic receptors active in cultured NG2$^+$ cells, and activation of P2Y$_1$ promotes their migration and inhibits their proliferation (Agresti et al., 2005a, 2005b).

The RNA-Seq transcriptome database largely confirms these findings (See Table 9), although the high expression of certain purinergic receptors by microglia, which are a 5% contamination in the NG2$^+$ cell sample, may affect the interpretation of these results. The database shows the highest expression of P2Y$_{12}$ (67 FPKM), but this receptor is expressed at extremely high levels in microglia ($\sim$330 FPKM), so this may reflect mRNA from microglia. Similarly, P2Y$_6$ barely surpassed the threshold in NG2$^+$ cells (2.7 FPKM), but it is expressed at much higher levels by microglia ($\sim$130 FPKM). Neither P2Y$_{12}$ nor P2Y$_6$ was found in cultured NG2$^+$ cells by Western blot (Agresti et al., 2005a). Setting those results aside, the purinergic receptors with the highest expression in NG2$^+$ cells are P2$\times_7$, P2$\times_4$, P2Y$_1$, and P2Y$_2$, which agrees with previous findings.

Recent studies indicate that NG2$^+$ cells migrate to sites of focal injury and contribute to the formation of the glial scar (Hughes et al., 2013). This behavior is reminiscent of that exhibited by microglia, which are attracted to injured cells by ATP-dependent activation of P2Y$_{12}$ receptors (Haynes et al., 2006). It remains to be determined if NG2$^+$ cells also use ATP as a chemoattractant signal to guide their movement to sites of injury to help promote repair.

3.3.6. Serotonin receptors

There are seven subtypes of serotonin receptors, 5-HT$_1$-5-HT$_7$. Other than 5-HT$_3$, which is ionotropic, all are metabotropic G protein-coupled receptors (Hoyer et al., 1994). Initial studies of cultured oligodendrocyte lineage cells (Belachew et al., 1998a; Karschin et al., 1994), glia in optic nerve explants

(Kriegler and Chiu, 1993) and corpus callosum slices (Bernstein et al., 1996), and human embryonic stem cell-derived oligodendrocyte progenitors (Schaumburg et al., 2008) reported that they responded with depolarization or Ca^{2+} elevation upon serotonin application. These responses were often limited to subpopulations of cells, and the identities of the cells were often not defined, leaving it unclear whether NG2$^+$ cells specifically express serotonin receptors. Furthermore, several other studies failed to find evidence of serotonin receptor expression in oligodendroglia in culture or in situ (Kastritsis and McCarthy, 1993; Maxishima et al., 2001).

A recent study used immunohistochemistry and Western blot to assess 5-HT$_{1A}$ and 5-HT$_{2A}$ receptor expression in cultured rat oligodendrocyte lineage cells, and expression of both receptors was found throughout the lineage (Fan et al., 2015). Intracellular Ca^{2+} elevation was also detected in response to serotonin, and chronic serotonin or 5-HT$_{2A}$ receptor agonist administration reduced differentiation of pure cultured NG2$^+$ cells and reduced myelination of axons in co-cultures. However, at present there have been no analyses of the in vivo expression of serotonin receptors by NG2$^+$ cells.

The RNA-Seq transcriptome database does not show significant expression of any serotonin receptor subunits in NG2$^+$ cells (Zhang et al., 2014). The subtype with highest expression is 5-HT$_{5A}$ receptor (1.4 FPKM, all other subunits$<$0.3 FPKM). There are species differences (rat vs. mouse) and age differences (P1 vs. P17) between these studies, which could explain the discrepancy, or it is possible that serotonin receptors are upregulated in culture conditions. Nevertheless, the expression of serotonin receptors on NG2$^+$ cells suggests that their behavior could be regulated by global changes in brain state, such as mood, appetite, and sleep. The potential effects of drugs affecting the serotonin system on NG2$^+$ cells are discussed below (see Discussion).

4. Discussion

NG2$^+$ cells are a dynamic population of glial cells that continue to divide, migrate and differentiate in the adult CNS, and perhaps not surprisingly, studies have revealed that there is significant variation in the physiological properties of these cells among different regions and between different developmental time points, and even between cells in the same brain region at the same age (Chittajallu et al., 2004; De Biase et al., 2011; Ge et al., 2006b; Kressin et al., 1995; Maldonado et al., 2011). For example, white matter NG2$^+$ cells have smaller capacitance, greater membrane resistance, and more depolarized RMP than gray matter NG2$^+$ cells (Chittajallu et al., 2004). Both physiological studies and RNA-Seq analysis reveal that channel and receptor expression change dramatically as NG2$^+$ cells differentiate into oligodendrocytes (De Biase et al., 2010; Zhang et al., 2014), and electrophysiological changes are associated with progression through the cell cycle in a variety of cell types (Blackiston et al., 2009; Sundelacruz et al., 2009), which may explain much of the observed variability. At present, the majority of physiological studies have been performed on cells in vitro or in tissue isolated from a small number of brain

regions (e.g. hippocampus) from young animals, so the full extent of the variation in channel and receptor gene expression remain to be defined. Nevertheless, some general patterns have emerged from analysis of existing datasets. It is clear that NG2^{+} cells express a wide variety of ion channels, including voltage-gated channels that are used by neurons to promote excitability and shape the waveform of synaptic potentials and action potentials. Given that NG2^{+} cells exhibit limited excitability and have a narrow range of voltage excursion under physiological conditions, some obvious questions are raised: why do these cells express these channels, and under what conditions are they activated?

NG2^{+} cells express ligand-gated receptors for a wide variety of neurotransmitters, which may provide a means for adjusting their behavior in response to changing patterns of neuronal activity. In the case of glutamate and GABA, it is known that these receptors are activated at defined synaptic junctions, in addition to spillover from nearby synapses (Bergles et al., 2000; Jabs et al., 2005; Kukley et al., 2008; Lin and Bergles, 2004; Vélez-Fort et al., 2010). However, under normal conditions, excitatory postsynaptic potentials (EPSPs) and K^{+}-mediated depolarizations result in small voltage changes at the cell body that are insufficient to induce substantial activation of voltage-gated channels (see Fig. 1E–F). There are several possible mechanisms by which this seeming inconsistency may be explained. First, while significant depolarization is required to activate large numbers of voltage-gated channels, channel opening is a probabilistic phenomenon, and some channel opening will occur even during smaller depolarizations. It is possible that such low levels of channel opening are sufficient to trigger intracellular changes in ion concentration that induce relevant downstream effects. Indeed, while depolarization of the cell body is limited, NG2^{+} cells have a complex morphology and extend many thin, highly branched processes. Ion influx into the small volume of these processes could affect transporter function or kinase activity, or even induce local depolarizations and activation of voltage-gated channels. Second, analysis of membrane potential changes of NG2^{+} cells have been performed exclusively in culture or in slices. No truly in vivo physiological recordings of NG2^{+} cells have been performed. As NG2^{+} cells respond rapidly to tissue damage (Hughes et al., 2013), it is possible that their properties are altered following preparation of brain slices and that they are capable of experiencing a larger dynamic voltage range in the intact CNS.

NG2^{+} cells in all brain regions that have been examined increase their resting conductance to K^{+} as the animal matures, resulting in a lower membrane resistance and an I–V relationship that approaches linearity (Kressin et al., 1995; Maldonado et al., 2013; Zhou et al., 2006). This change has important implications for activation of voltage-gated conductances, as larger currents would be required to bring the membrane potential of the cell into a range where substantial gating is possible. However, if leak conductances are absent from the fine processes, they may experience much greater depolarization, as well as larger concentration changes. Such changes are not easily detectable by whole-cell patch clamp recording or the use of voltage-sensitive dyes, but new methods such as the use of genetically-encoded voltage and Ca^{2+} indicators may allow exploration of the voltage excursions experienced by these domains. NG2^{+}

cells expressing fluorescent proteins in superficial cortical layers can be imaged through cranial windows (Hughes et al., 2013), potentially allowing analysis of NG2^{+} cell physiology in vivo and even correlation of their responses with animal behavior.

The expression of neuromodulator receptors has mainly been demonstrated by exogenous application of agonists in culture or in brain slices. Spontaneous activation of these receptors in situ or in vivo has not yet been reported. Unlike glutamate and GABA, neuromodulators such as norepinephrine and serotonin are typically released from varicosities by long-range projection neurons, potentially affecting all neurons and glia with relevant receptors in a broad area. One exception is ATP, which can be released locally by astrocytes and from synaptic vesicles (Hamilton et al., 2010; Jo and Role, 2002; Jo and Schlichter, 1999; Pankratov et al., 2006). Although mRNAs encoding neuromodulatory receptors were present in low abundance, the high signal amplification exhibited by these receptors may enable a few receptors to exert profound alterations in NG2^{+} cells. Neuromodulator release is associated with global changes in brain state, such as mood, attention, and sleep. The regulation of NG2^{+} cell behavior by these state changes is largely unexplored, but the expression of neuromodulator receptors suggests that they have the potential to be affected and may contribute to the plastic changes in neural circuits induced by these neurotransmitters.

The RNA-Seq transcriptome database has provided the first comprehensive analysis of mRNA expression within this population of glial cells, enabling comparisons of gene expression between glial cell types and neurons. At present, this dataset is only a snapshot, revealing the properties of NG2^{+} cells from a single time point and brain region. Thus, referencing these values to physiological data acquired across a range of conditions, ages and brain regions is likely to reveal inconsistencies, as noted above. Furthermore, the RNA-Seq analysis only measures mRNA levels; it is possible that protein expression from some mRNAs may be tightly regulated and therefore may not correlate well with functional protein expression. Other methods such as immunohistochemistry and electrophysiology combined with specific pharmacology, which are not as amenable to high-throughput analysis, will be required to corroborate mRNA levels with the expression of functional channels and receptors.

NG2^{+} cells undergo dramatic morphological and physiological changes upon differentiation into oligodendrocytes. To determine how this maturation changes the complement of ion channels and receptors expressed, we identified 290 genes from the RNA-Seq database that encode subunits of plasma membrane ion channels or known neurotransmitter receptors (including all genes listed in Tables 1–9, in addition to other genes from the same categories expressed from 0.1-2 FPKM). The overall expression levels of these genes were significantly different among NG2^{+} cells, premyelinating oligodendrocytes, and mature oligodendrocytes (see Fig. 2) (two-sample Kolmogorov–Smirnov test; NG2^{+} cells vs. premyelinating oligodendrocytes: $D=0.17$, $Prob>|D|=2.8 \times 10^{-4}$; NG2^{+} cells vs. mature oligodendrocytes: $D=0.3$, $Prob>|D|=6.1 \times 10^{-12}$; Premyelinating oligodendrocytes vs. mature oligodendrocytes: $D=0.13$, $Prob>|D|=0.01$). At any threshold of significance greater than 0.1 FPKM, NG2^{+} cells express a much larger group of channel and receptor genes than more
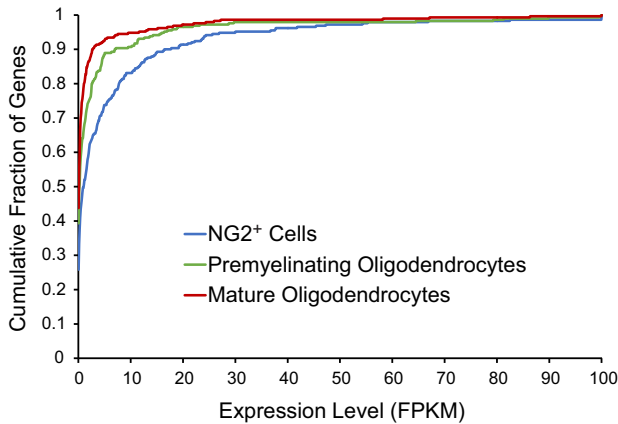
Fig. 2 – Expression of ion channel and neurotransmitter receptor genes at different stages in the oligodendrocyte lineage. Cumulative fraction of genes expressed at or below 0.1–100 FPKM in NG2$^+$ cells (blue), premyelinating oligodendrocytes (green), and mature oligodendrocytes (red) of 290 genes identified from the RNA-Seq database (Zhang et al., 2014) representing ion channels and neurotransmitter receptors. This pool includes all genes listed in Tables 1–9, in addition to other genes from the same categories expressed from 0.1–2 FPKM. Bin size=0.1 FPKM. The distributions are significantly different from one another in pairwise comparisons (two-sample Kolmogorov–Smirnov test; NG2$^+$ cells vs. premyelinating oligodendrocytes: $D=0.17$, $Prob>|D|=2.8 \times 10^{-4}$; NG2$^+$ cells vs. mature oligodendrocytes: $D=0.3$, $Prob>|D|=6.1 \times 10^{-12}$; Premyelinating oligodendrocytes vs. mature oligodendrocytes: $D=0.13$, $Prob>|D|=0.01$). The expression of ion channel and neurotransmitter receptor genes is significantly reduced as NG2$^+$ cells differentiate into oligodendrocytes.

differentiated cells. For example, 120/290 genes (41%) are expressed at ≥ 2 FPKM in NG2$^+$ cells, while only 74/290 (26%) and 40/290 (14%) are expressed at that level in premyelinating and mature oligodendrocytes, respectively. This dramatic difference in the diversity of ion channel and receptor expression suggests that NG2$^+$ cells are uniquely positioned within the oligodendrocyte lineage to be influenced by diverse forms of neural activity.

4.1.   NG2$^+$ cells as drug targets

The role of glial cells in neuropsychiatric disease is an area of increasing interest (for a comprehensive review, see (Di Benedetto and Rupprecht, 2013) and Joel Levine's chapter in this issue). Many therapeutics used to treat neuropsychiatric diseases target ion channels and neurotransmitter receptors in the brain. Although, the intended targets are often neurons, NG2$^+$ cells express many 'neuronal' genes that will also bind to these drugs; thus, these cells could contribute to both unexpected side effects and therapeutic benefits of these agents.

Among the most common targets of psychiatric drugs and drugs of abuse are the dopamine and serotonin systems.

Olanzapine, an atypical antipsychotic, and haloperidol, a typical antipsychotic, have been shown to enhance cell proliferation and reduce differentiation of NG2$^+$ cells in vitro (Kimoto et al., 2011; Niu et al., 2010). Antipsychotics are thought to function primarily by antagonizing D_2/D_3 and 5-HT_{2A} receptors, though they are able to bind to a wide variety of other neuromodulator receptors as well (Kusumi et al., 2015). The D_3 and the 5-HT_{2A} receptor have been found in cultured NG2$^+$ cells, but not in the acutely isolated cortical NG2$^+$ cells of the RNA-Seq database (see Sections 3.3.3 and 3.3.6). In a concurrent in vivo study, it was found that administration of haloperidol to adult mice resulted in an increase in the density of NG2$^+$ cells in the brain with no increase in the number of oligodendrocytes, supporting the in vivo relevance of these finding (Wang et al., 2010). It has also been shown that perinatal exposure to the selective serotonin reuptake inhibitor (SSRI) citalopram impairs oligodendrocyte development and myelination in the corpus callosum of rats, (Simpson et al., 2011) suggesting that the serotonin system is relevant to NG2$^+$ cells in vivo.

In addition to therapeutics, drugs of abuse also frequently target ion channels and receptors, and may affect NG2$^+$ cells in ways that are not well understood. One important example is nicotine: NG2$^+$ cells express calcium-permeable nicotinic acetylcholine receptors in vivo (Vélez-Fort et al., 2009) (see Section 3.3.2), and in vitro application of nicotine results in intracellular calcium oscillations in NG2$^+$ cells (Rogers et al., 2001). At typical concentrations reached in the brain during smoking, application of nicotine to acute brain slices does not evoke an electrical response in NG2$^+$ cells, but does desensitize the receptors, potentially interfering with their normal function (Vélez-Fort et al., 2009). However, the potential effects of most drugs on NG2$^+$ cells have not been investigated, and dissociating the direct effects of these compounds on these cells versus indirect effects on neurons will require selective genetic manipulation of NG2$^+$ cells, as in many cases their receptors cannot be selectively interrogated pharmacologically. As we increase our understanding of the contribution of glial cells to neuropsychiatric disease and develop new therapeutic strategies, it will be important to consider the potential effects on non-neuronal cells. NG2$^+$ cells in particular, with their broad expression of 'neuronal' genes, are likely to be direct targets for both therapeutics and drugs of abuse.

Acknowledgments

REFERENCES

Abbracchio, M.P., Burnstock, G., Boeynaems, J.-M., Barnard, E.A., Boyer, J.L., Kennedy, C., Knight, G.E., Fumagalli, M., Gachet, C., Jacobson, K.A., Weisman, G.A., 2006. International Union of

Pharmacology LVIII: update on the P2Y G protein-coupled nucleotide receptors: from molecular mechanisms and pathophysiology to therapy. Auton. Neurosci. 58, 281–341, http://dx.doi.org/10.1124/pr.58.3.3.281.

Abiraman, K., Pol, S.U., O'Bara, M.A., Chen, G.-D., Khaku, Z.M., Wang, J., Thorn, D.A., Vedia, B.H., Ekwegbalu, E.C., Li, J.-X., Salvi, R.J., Sim, F.J., 2015. Anti-muscarinic adjunct therapy accelerates functional human oligodendrocyte repair. J. Neurosci. 35, 3676–3688, http://dx.doi.org/10.1523/JNEUROSCI.3510-14.2015.

Agresti, C., Meomartini, M.E., Amadio, S., Ambrosini, E., Serafini, B., Franchini, L., Volonté, C., Aloisi, F., Visentin, S., 2005a. Metabotropic P2 receptor activation regulates oligodendrocyte progenitor migration and development. Glia 50, 132–144, http://dx.doi.org/10.1002/glia.20160.

Agresti, C., Meomartini, M.E., Amadio, S., Ambrosini, E., Volonté, C., Aloisi, F., Visentin, S., 2005b. ATP regulates oligodendrocyte progenitor migration, proliferation, and differentiation: Involvement of metabotropic P2 receptors. Brain Res. Rev. 48, 157–165, http://dx.doi.org/10.1016/j.brainresrev.2004.12.005.

Aguirre, A., Gallo, V., 2004. Postnatal neurogenesis and gliogenesis in the olfactory bulb from NG2-expressing progenitors of the subventricular zone. J. Neurosci. 24, 10530–10541, http://dx.doi.org/10.1523/JNEUROSCI.3572-04.2004.

Akopian, G., Kressin, K., Derouiche, A., Steinhäuser, C., 1996. Identified glial cells in the early postnatal mouse hippocampus display different types of Ca2+ currents. Glia 17, 181–194, http://dx.doi.org/10.1002/(SICI)1098-1136(199607)17:3<181::AID-GLIA1>3.0.CO;2-4.

Attali, B., Wang, N., Kolot, A., Sobko, A., Cherepanov, V., Soliven, B., 1997. Characterization of delayed rectifier Kv channels in oligodendrocytes and progenitor cells. J. Neurosci. 17, 8234–8245.

Balia, M., Vélez-Fort, M., Passlick, S., Schäfer, C., Audinat, E., Steinhäuser, C., Seifert, G., Angulo, M.C., 2015. Postnatal down-regulation of the GABAA receptor γ2 subunit in neocortical NG2 cells accompanies synaptic-to-extrasynaptic switch in the GABAergic transmission mode. Cereb. Cortex 25, 1114–1123, http://dx.doi.org/10.1093/cercor/bht309.

Barres, B.A., Koroshetz, W.J., Swartz, K.J., Chun, L.L.Y., Corey, D.P., 1990. Ion channel expression by white matter glia: the O-2A glial progenitor cell. Neuron 4, 507–524, http://dx.doi.org/10.1016/0896-6273(90)90109-S.

Beaulieu, J.-M., Gainetdinov, R.R., 2011. The physiology, signaling, and pharmacology of dopamine receptors. Pharmacol. Rev. 63, 182–217, http://dx.doi.org/10.1124/pr.110.002642.182.

Bekar, L.K., Loewen, M.E., Cao, K., Sun, X., Leis, J., Wang, R., Forsyth, G.W., Walz, W., 2005. Complex expression and localization of inactivating Kv channels in cultured hippocampal astrocytes. J. Neurophysiol. 93, 1699–1709, http://dx.doi.org/10.1152/jn.00850.2004.

Belachew, S., Chittajallu, R., Aguirre, A.A., 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, http://dx.doi.org/10.1083/jcb.200210110.

Belachew, S., Malgrange, B., Rigo, J.-M., Rogister, B., Coucke, P., Mazy-Servais, C., Moonen, G., 1998a. Developmental regulation of neuroligand-induced responses in cultured oligodendroglia. NeuroReport 9, 973–980, http://dx.doi.org/10.1097/00001756-199804200-00004.

Belachew, S., Malgrange, B., Rigo, J.-M.M., Rogister, B., Leprince, P., Hans, G., Nguyen, L., Moonen, G., 2000. Glycine triggers an intracellular calcium influx in oligodendrocyte progenitor cells which is mediated by the activation of both the ionotropic glycine receptor and Na+-dependent transporters. Eur. J. Neurosci. 12, 1924–1930.

Belachew, S., Rogister, B., Rigo, J.-M., Malgrange, B., Mazy-Servais, C., Xhauflaire, G., Coucke, P., Moonen, G., 1998b. Cultured oligodendrocyte progenitors derived from cerebral cortex express a glycine receptor which is pharmacologically distinct from the neuronal isoform. Eur. J. Neurosci. 10, 3556–3564, http://dx.doi.org/10.1046/j.1460-9568.1998.00369.x.

Benarroch, E.E., 2012. GABA B receptors: Structure, functions, and clinical implications. Neurology 78, 578–584, http://dx.doi.org/10.1212/WNL.0b013e318247cd03.

Berger, T., Schnitzer, J., Kettenmann, H., 1991. Developmental changes in the membrane current pattern, K+ buffer capacity, and morphology of glial cells in the corpus callosum slice. J. Neurosci. 11, 3008–3024.

Berger, T., Schnitzer, J., Orkand, P.M., Kettenmann, H., 1992a. Sodium and calcium currents in glial cells of the mouse corpus callosum slice. Eur. J. Neurosci. 4, 1271–1284, http://dx.doi.org/10.1111/j.1460-9568.1992.tb00153.x.

Berger, T., Walz, W., Schnitzer, J., Kettenmann, H., 1992b. GABA- and glutamate-activated currents in glial cells of the mouse corpus callosum slice. J. Neurosci. Res. 31, 21–27, http://dx.doi.org/10.1002/jnr.490310104.

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.

Bernstein, M., Lyons, S.A., Möller, T., Kettenmann, H., 1996. Receptor-mediated calcium signalling in glial cells from mouse corpus callosum slices. J. Neurosci. Res. 46, 152–163, http://dx.doi.org/10.1002/(SICI)1097-4547(19961015)46:2<152::AID-JNR3>3.0.CO;2-G.

Blackiston, D.J., McLaughlin, K.A., Levin, M., 2009. Bioelectric controls of cell proliferation: ion channels, membrane voltage and the cell cycle. Cell Cycle 8, 3519–3528, http://dx.doi.org/10.4161/cc.8.21.9888.

Blanz, J., Schweizer, M., Auberson, M., Maier, H., Muenscher, A., Hübner, C.A., Jentsch, T.J., 2007. Leukoencephalopathy upon disruption of the chloride channel ClC-2. J. Neurosci. 27, 6581–6589, http://dx.doi.org/10.1523/JNEUROSCI.0338-07.2007.

Bongarzone, E.R., Howard, S.G., Schonmann, V., Campagnoni, A.T., 1998. Identification of the dopamine D3 receptor in oligodendrocyte precursors: potential role in regulating differentiation and myelin formation. J. Neurosci. 18, 5344–5353.

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, http://dx.doi.org/10.1016/0306-4522(94)90012-4.

Borges, K., Wolswijk, G., Ohlemeyer, C., Kettenmann, H., 1995. Adult rat optic nerve oligodendrocyte progenitor cells express a distinct repertoire of voltage- and ligand-gated ion channels. J. Neurosci. Res. 40, 591–605, http://dx.doi.org/10.1002/jnr.490400504.

Bormann, J., Rundstrom, N., 1993. Residues within transmembrane segment M2 determine chloride conductance of glycine receptor homo- and. EMBO J. 12, 3729–3737.

Burgard, E.C., Tietz, E.I., Neelands, T.R., MacDonald, R.L., 1996. Properties of recombinant gamma-aminobutyric acid A receptor isoforms containing the alpha 5 subunit subtype. Mol. Pharmacol. 50, 119–127..

Burnstock, G., Fredholm, B.B., Verkhratsky, A., 2011. Adenosine and ATP receptors in the brain. Curr. Top. Med. Chem. 11, 973–1011, http://dx.doi.org/10.2174/156802611795347627.

Butt, A.M., Kalsi, A.S., 2006. Inwardly rectifying potassium channels (Kir) in central nervous system glia: a special role for Kir4.1 in glial functions. J. Cell. Mol. Med. 10, 33–44, http://dx.doi.org/10.1111/j.1582-4934.2006.tb00289.x.

Buttigieg, J., Eftekharpour, E., Karimi-Abdolrezaee, S., Fehlings, M.G., 2011. Molecular and electrophysiological evidence for the

expression of BK channels in oligodendroglial precursor cells. Eur. J. Neurosci. 34, 538–547, http://dx.doi.org/10.1111/j.1460-9568.2011.07789.x.

Bylund, D.B., Eikenberg, D.C., Hieble, J.P., Langer, S.Z., Lefkowitz, R.J., Minneman, K.P., Molinoff, P.B., Ruffalo, R.R., Trendelenburg, U., 1994. IV. International Union of Pharmacology nomenclature of adrenoceptors. Pharmacol. Rev. 46, 121–136.

Cahoy, J.D., Emery, B., Kaushal, A., Foo, L.C., Zamanian, J.L., Christopherson, K.S., Xing, Y., Lubischer, J.L., Krieg, P.A., Krupenko, S.A., Thompson, W.J., Barres, B.A., 2008. A transcriptome database for astrocytes, neurons, and oligodendrocytes: a new resource for understanding brain development and function. J. Neurosci. 28, 264–278, http://dx.doi.org/10.1523/JNEUROSCI.4178-07.2008.

Catterall, W.A., Goldin, A.L., Waxman, S.G., 2005a. International Union of Pharmacology. XLVII. Nomenclature and structure-function relationships of voltage-gated sodium channels. Pharmacol. Rev. 57, 397–409, http://dx.doi.org/10.1124/pr.57.4.4.

Catterall, W.A., Perez-Reyes, E., Snutch, T.P., Striessnig, J., 2005b. International Union of Pharmacology. XLVIII. Nomenclature and structure-function relationships of voltage-gated calcium channels. Pharmacol. Rev. 57, 411–425, http://dx.doi.org/10.1124/pr.57.4.5.

Caulfield, M.P., Birdsall, N.J., 1998. International Union of Pharmacology. XVII. Classification of muscarinic acetylcholine receptors. Pharmacol. Rev. 50, 279–290.

Chang, S.Y., Zagha, E., Kwon, E.S., Ozaita, A., Bobik, M., Martone, M.E., Ellisman, M.H., Heintz, N., Rudy, B., 2007. Distribution of Kv3.3 potassium channel subunits in distinct neuronal populations of mouse brain. J. Comp. Neurol. 502, 953–972, http://dx.doi.org/10.1002/cne.21353.

Cheli, V.T., Santiago González, D.A., Spreuer, V., Paez, P.M., 2015. Voltage-gated Ca++ entry promotes oligodendrocyte progenitor cell maturation and myelination in vitro. Exp. Neurol. 265, 69–83, http://dx.doi.org/10.1016/j.expneurol.2014.12.012.

Chever, O., Djukic, B., McCarthy, K.D., Amzica, F., 2010. Implication of Kir4.1 channel in excess potassium clearance: an in vivo study on anesthetized glial-conditional Kir4.1 knockout mice. J. Neurosci. 30, 15769–15777, http://dx.doi.org/10.1523/JNEUROSCI.2078-10.2010.

Chittajallu, R., Aguirre, A., Gallo, V., 2004. NG2-positive cells in the mouse white and grey matter display distinct physiological properties. J. Physiol. 561, 109–122, http://dx.doi.org/10.1113/jphysiol.2004.074252.

Chittajallu, R., Chen, Y., Wang, H., Yuan, X., Ghiani, C.A., Heckman, T., McBain, C.J., Gallo, V., 2002. Regulation of Kv1 subunit expression in oligodendrocyte progenitor cells and their role in G1/S phase progression of the cell cycle. Proc. Natl. Acad. Sci. USA 99, 2350–2355, http://dx.doi.org/10.1073/pnas.042698399.

Clapham, D.E., Julius, D., Montell, C., Schultz, G., 2005. International Union of Pharmacology. XLIX. Nomenclature and structure-function relationships of transient receptor potential channels. Pharmacol. Rev. 57, 427–450, http://dx.doi.org/10.1124/pr.57.4.6.1.

Clarke, L.E., Young, K.M., Hamilton, N.B., Li, H., Richardson, W.D., Attwell, D., 2012. Properties and fate of oligodendrocyte progenitor cells in the corpus callosum, motor cortex, and piriform cortex of the mouse. J. Neurosci. 32, 8173–8185, http://dx.doi.org/10.1523/JNEUROSCI.0928-12.2012.

Cohen, R.I., Almazan, G., 1994. Rat oligodendrocytes express muscarinic receptors coupled to phosphoinositide hydrolysis and adenylyl cyclase. Eur. J. Neurosci. 6, 1213–1224, http://dx.doi.org/10.1111/j.1460-9568.1994.tb00620.x.

Cohen, R.I., Almazan, G., 1993. Norepinephrine-stimulated PI hydrolysis in oligodendrocytes is mediated by alpha 1A-adrenoceptors. NeuroReport 4, 1115–1118.

Cohen, R.I., Molina-Holgado, E., Almazan, G., 1996. Carbachol stimulates c-fos expression and proliferation in oligodendrocyte progenitors. Mol. Brain Res. 43, 193–201, http://dx.doi.org/10.1016/S0169-328X(96)00176-3.

Coppi, E., Cellai, L., Maraula, G., Pugliese, A.M., Pedata, F., 2013. Adenosine A2A receptors inhibit delayed rectifier potassium currents and cell differentiation in primary purified oligodendrocyte cultures. Neuropharmacology 73, 301–310, http://dx.doi.org/10.1016/j.neuropharm.2013.05.035.

Cui, Q.L., Fogle, E., Almazan, G., 2006. Muscarinic acetylcholine receptors mediate oligodendrocyte progenitor survival through Src-like tyrosine kinases and PI3K/Akt pathways. Neurochem. Int. 48, 383–393, http://dx.doi.org/10.1016/j.neuint.2005.11.014.

Dal Ben, D., Buccioni, M., Lambertucci, C., Marucci, G., Thomas, A., Volpini, R., 2015. Purinergic P2X receptors: Structural models and analysis of ligand-target interaction. Eur. J. Med. Chem. 89, 561–580, http://dx.doi.org/10.1016/j.ejmech.2014.10.071.

De Biase, L.M., Kang, S.H., Baxi, E.G., Fukaya, M., Pucak, M.L., Mishina, M., Calabresi, P.A., Bergles, D.E., 2011. NMDA receptor signaling in oligodendrocyte progenitors is not required for oligodendrogenesis and myelination. J. Neurosci. 31, 12650–12662, http://dx.doi.org/10.1523/JNEUROSCI.2455-11.2011.

De Biase, L.M., Nishiyama, A., Bergles, D.E., 2010. Excitability and synaptic communication within the oligodendrocyte lineage. J. Neurosci. 30, 3600–3611, http://dx.doi.org/10.1523/JNEUROSCI.6000-09.2010.

Deng, W., Neve, R.L., Rosenberg, P.A., Volpe, J.J., Jensen, F.E., 2006. α-amino-3-hydroxy-5-methyl-4-isoxazole propionate receptor subunit composition and cAMP-response element-binding protein regulate oligodendrocyte excitotoxicity. J. Biol. Chem. 281, 36004–36011, http://dx.doi.org/10.1074/jbc.M606459200.

Deng, W., Wang, H., Rosenberg, P.A., Volpe, J.J., Jensen, F.E., 2004. Role of metabotropic glutamate receptors in oligodendrocyte excitotoxicity and oxidative stress. Proc. Natl. Acad. Sci. USA 101, 7751–7756, http://dx.doi.org/10.1073/pnas.0307850101.

Deshmukh, V.A., Tardif, V., Lyssiotis, C.A., Green, C.C., Kerman, B., Kim, H.J., Padmanabhan, K., Swoboda, J.G., Ahmad, I., Kondo, T., Gage, F.H., Theofilopoulos, A.N., Lawson, B.R., Schultz, P.G., Lairson, L.L., 2013. A regenerative approach to the treatment of multiple sclerosis. Nature 502, 327–332, http://dx.doi.org/10.1038/nature12647.

Di Benedetto, B., Rupprecht, R., 2013. Targeting glia cells: novel perspectives for the treatment of neuropsychiatric diseases. Curr. Neuropharmacol. 11, 171–185, http://dx.doi.org/10.2174/1570159X11311020004.

Djukic, B., Casper, K.B., Philpot, B.D., Chin, L.-S., McCarthy, K.D., 2007. Conditional knock-out of Kir4.1 leads to glial membrane depolarization, inhibition of potassium and glutamate uptake, and enhanced short-term synaptic potentiation. J. Neurosci. 27, 11354–11365, http://dx.doi.org/10.1523/JNEUROSCI.0723-07.2007.

Du, J., Tao-Cheng, J.-H., Zerfas, P., McBain, C.J., 1998. The K+ channel, Kv2.1, is apposed to astrocytic processes and is associated with inhibitory postsynaptic membranes in hippocampal and cortical principal neurons and inhibitory interneurons. Neuroscience 84, 37–48, http://dx.doi.org/10.1016/S0306-4522(97)00519-8.

Fan, L.-W., Bhatt, A., Tien, L.-T., Zheng, B., Simpson, K.L., Lin, R.C.S., Cai, Z., Kumar, P., Pang, Y., 2015. Exposure to serotonin adversely affects oligodendrocyte development and myelination in vitro. J. Neurochem.http://dxdoi.org/10.1111/jnc.12988 n/a–n/a.

Fannon, J., Tarmier, W., Fulton, D., 2015. Neuronal Activity and AMPA-type glutamate receptor activation regulates the

morphological development of oligodendrocyte precursor cells. Glia 63, 1021–1035, http://dx.doi.org/10.1002/glia.22799.

Farrant, M., Nusser, Z., 2005. Variations on an inhibitory theme: phasic and tonic activation of GABA(A) receptors. Nat. Rev. Neurosci. 6, 215–229, http://dx.doi.org/10.1038/nrn1625.

Feliciangeli, S., Chatelain, F.C., Bichet, D., Lesage, F., 2014. The family of K2P channels: salient structural and functional properties. J. Physiol. 0http://dxdoi.org/10.1113/jphysiol.2014.287268 n/a–n/a.

Feliciangeli, S., Tardy, M.P., Sandoz, G., Chatelain, F.C., Warth, R., Barhanin, J., Bendahhou, S., Lesage, F., 2010. Potassium channel silencing by constitutive endocytosis and intracellular sequestration. J. Biol. Chem. 285, 4798–4805, http://dx.doi.org/10.1074/jbc.M109.078535.

Fleig, A., Chubanov, V., 2014. TRPM7. Handb. Exp. Pharmacol. 222 (222), 521–546, http://dx.doi.org/10.1007/978-3-642-54215-2_21.

Fredholm, B.B., IJzerman, A.P., Jacobson, K.A., Klotz, K.-N., Linden, J., 2001. International Union of Pharmacology. XXV. Nomenclature and classification of adenosine receptors. Pharmacol. Rev. 53, 527–552.

Fredholm, B.B., IJzerman, A.P., Jacobson, K.A., Linden, J., Ller Mller, C.E., 2011. International Union of Basic and Clinical Pharmacology. LXXXI. Nomenclature and classification of adenosine receptors-an update. Pharmacol. Rev. 63, 1–34, http://dx.doi.org/10.1124/pr.110.003285.

Fulton, D., Paez, P.M., Fisher, R., Handley, V., Colwell, C.S., Campagnoni, A.T., 2010. Regulation of L-type Ca++ currents and process morphology in white matter oligodendrocyte precursor cells by golli-myelin proteins. Glia 58, 1292–1303, http://dx.doi.org/10.1002/glia.21008.

Fusco, F.R., Martorana, A., Giampà, C., de March, Z., Vacca, F., Tozzi, A., Longone, P., Piccirilli, S., Paolucci, S., Sancesario, G., Mercuri, N.B., Bernardi, G., 2004. Cellular localization of TRPC3 channel in rat brain: Preferential distribution to oligodendrocytes. Neurosci. Lett. 365, 137–142, http://dx.doi.org/10.1016/j.neulet.2004.04.070.

Gallo, V., Zhou, J.-M., McBain, C.J., Wright, P.W., 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.

Ge, S., Goh, E.L.K., Sailor, K.A., Kitabatake, Y., Ming, G., Song, H., 2006a. GABA regulates synaptic integration of newly generated neurons in the adult brain. Nature 439, 589–593, http://dx.doi.org/10.1038/nature04404.

Ge, W.P., Yang, X.-J., Zhang, Z., Wang, H.-K., Shen, W., Deng, Q.-D., Duan, S., 2006b. Long-term potentiation of neuron-glia synapses mediated by Ca2+-permeable AMPA receptors. Science 80- (312), 1533–1537.

Ghiani, C.A., Eisen, A.M., Yuan, X., DePinho, R.A., McBain, C.J., Gallo, V., 1999. Neurotransmitter receptor activation triggers p27(Kip1)and p21(CIP1) accumulation and G1 cell cycle arrest in oligodendrocyte progenitors. Development 126, 1077–1090.

Gingrich, K.J., Roberts, W.A., Kass, R.S., 1995. Dependence of the GABA(A) receptor gating kinetics on the alpha-subunit isoform: implications for structure-function relations and synaptic transmission. J. Physiol. 489, 529–543.

Goldman, S., 2003. Glia as neural progenitor cells. Trends Neurosci. 26, 590–596, http://dx.doi.org/10.1016/j.tins.2003.09.011.

Goldstein, P.A., Elsen, F.P., Ying, S.-W., Ferguson, C., Homanics, G.E., Harrison, N.L., 2002. Prolongation of hippocampal miniature inhibitory postsynaptic currents in mice lacking the GABA(A) receptor alpha1 subunit. J. Neurophysiol. 88, 3208–3217, http://dx.doi.org/10.1152/jn.00885.2001.

Griffon, N., Bu, C., Nicke, A., Kuhse, J., 1999. Molecular determinants of glycine receptor subunit assembly. EMBO J. 18, 4711–4721.

Gudz, T.I., Komuro, H., Macklin, W.B., 2006. Glutamate stimulates oligodendrocyte progenitor migration mediated via an alphav integrin/myelin proteolipid protein complex. J. Neurosci. 26, 2458–2466, http://dx.doi.org/10.1523/JNEUROSCI.4054-05.2006.

Gupta, R.K., Kanungo, M., 2013. Glial molecular alterations with mouse brain development and aging: Up-regulation of the Kir4.1 and aquaporin-4. Age 35, 59–67, http://dx.doi.org/10.1007/s11357-011-9330-5.

Gutman, G.A, Chandy, K.G., Grissmer, S., Lazdunski, M., McKinnon, D., Pardo, L.A., Robertson, G.A., Rudy, B., Sanguinetti, M.C., Sẗhmer, W., Wang, X., 2005. International Union of Pharmacology. LIII. Nomenclature and molecular relationships of voltage-gated potassium channels. Pharmacol. Rev. 57, 473–508, http://dx.doi.org/10.1124/pr.57.4.10.1.

Haberlandt, C., Derouiche, A., Wyczynski, A., Haseleu, J., Pohle, J., Karram, K., Trotter, J., Seifert, G., Frotscher, M., Steinhäuser, C., Jabs, R., 2011. Gray matter NG2 cells display multiple Ca2+-signaling pathways and highly motile processes. PLoS One 6, e17575, http://dx.doi.org/10.1371/journal.pone.0017575.

Hamilton, N.B., Vayro, S., Wigley, R., Butt, A.M., 2010. Axons and astrocytes release ATP and glutamate to evoke calcium signals in NG2-glia. Glia 58, 66–79, http://dx.doi.org/10.1002/glia.20902.

Haynes, S.E., Hollopeter, G., Yang, G., Kurpius, D., Dailey, M.E., Gan, W.-B., Julius, D., 2006. The P2Y12 receptor regulates microglial activation by extracellular nucleotides. Nat. Neurosci. 9, 1512–1519, http://dx.doi.org/10.1038/nn1805.

He, M., McCarthy, K.D., 1994. Oligodendroglial signal transduction systems are developmentally regulated. J. Neurochem. 63, 501–508, http://dx.doi.org/10.1046/j.1471-4159.1994.63020501.x.

Hibino, H., Fujita, A., Iwai, K., Yamada, M., Kurachi, Y., 2004. Differential assembly of inwardly rectifying K+ channel subunits, Kir4.1 and Kir5.1, in brain astrocytes. J. Biol. Chem. 279, 44065–44073, http://dx.doi.org/10.1074/jbc.M405985200.

Hirano, T., 2012. Glutamate-receptor-like molecule GluRδ2 involved in synapse formation at parallel fiber-Purkinje neuron synapses. Cerebellum 11, 71–77, http://dx.doi.org/10.1007/s12311-010-0170-0.

Hoffmann, A., Grimm, C., Kraft, R., Goldbaum, O., Wrede, A., Nolte, C., Hanisch, U.K., Richter-Landsberg, C., Brück, W., Kettenmann, H., Harteneck, C., 2010. TRPM3 is expressed in sphingosine-responsive myelinating oligodendrocytes. J. Neurochem. 114, 654–665, http://dx.doi.org/10.1111/j.1471-4159.2010.06644.x.

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.

Hoyer, D., Clarke, D.E., Fozard, J.R., Hartig, P.R., Martin, G.R., Mylecharane, E.J., Saxena, P.R., Humphrey, P.P., 1994. nternational Union of Pharmacology classification of receptors for 5-hydroxytryptamine (Serotonin). Pharmacol. Rev. 46, 157–203.

Hughes, E.G., Kang, S.H., Fukaya, M., Bergles, D.E., 2013. Oligodendrocyte progenitors balance growth with self-repulsion to achieve homeostasis in the adult brain. Nat. Neurosci. 16, 668–676, http://dx.doi.org/10.1038/nn.3390.

Ikonomidou, C., Bosch, F., Miksa, M., Bittigau, P., Vöckler, J., Dikranian, K., Tenkova, T.I., Stefovska, V., Turski, L., Olney, J.W., 1999. Blockade of NMDA receptors and apoptotic neurodegeneration in the developing brain. Science 283, 70–74, http://dx.doi.org/10.1126/science.283.5398.70.

Itoh, T., Beesley, J., Itoh, A., Cohen, A.S., Kavanaugh, B., Coulter, D.A., Grinspan, J.B., Pleasure, D., 2002. AMPA glutamate receptor-mediated calcium signaling is transiently enhanced during development of oligodendrocytes. J. Neurochem. 81, 390–402, http://dx.doi.org/10.1046/j.1471-4159.2002.00866.x.

Itoh, T., Reddy, U.R., Stern, J.L., Chen, M., Itoh, A., Pleasure, D., 2000. Diminished calcium homeostasis and increased susceptibility to excitotoxicity of JS 3/16 progenitor cells after differentiation

to oligodendroglia. Glia 31, 165–180, http://dx.doi.org/10.1002/1098-1136(200008)31:2<165::AID-GLIA80>3.0.CO;2-#.

Jabs, R., Kirchhoff, F., Kettenmann, H., Steinhäuser, C., 1994. Kainate activates Ca2+-permeable glutamate receptors and blocks voltage-gated K+ currents in glial cells of mouse hippocampal slices. Eur. J. Physiol. 426, 310–319, http://dx.doi.org/10.1007/BF00374787.

Jabs, R., Pivneva, T., Hüttmann, K., Wyczynski, A., Nolte, C., Kettenmann, H., Steinhäuser, C., 2005. Synaptic transmission onto hippocampal glial cells with hGFAP promoter activity. J. Cell Sci. 118, 3791–3803, http://dx.doi.org/10.1242/jcs.02515.

Jansson, L.C., Akerman, K.E., 2014. The role of glutamate and its receptors in the proliferation, migration, differentiation and survival of neural progenitor cells. J. Neural Transm. 121, 819–836, http://dx.doi.org/10.1007/s00702-014-1174-6.

Jentsch, T.J., 2008. CLC chloride channels and transporters: from genes to protein structure, pathology and physiology. Crit. Rev. Biochem. Mol. Biol. 43, 3–36 1080/10409230701829110.

Jo, Y.-H., Role, L.W., 2002. Coordinate release of ATP and GABA at in vitro synapses of lateral hypothalamic neurons. J. Neurosci. 22, 4794–4804.

Jo, Y.-H., Schlichter, R., 1999. Synaptic corelease of ATP and GABA in cultured spinal neurons. Nat. Neurosci. 2, 241–245, http://dx.doi.org/10.1038/6344.

Kalsi, A.S., Greenwood, K., Wilkin, G.P., Butt, A.M., 2004. Kir4.1 expression by astrocytes and oligodendrocytes in CNS white matter: a developmental study in the rat optic nerve. J. Anat. 204, 475–485, http://dx.doi.org/10.1111/j.0021-8782.2004.00288.x.

Káradóttir, R., Cavelier, P., Bergersen, L.H., Attwell, D., 2005. NMDA receptors are expressed in oligodendrocytes and activated in ischaemia. Nature 438, 1162–1166, http://dx.doi.org/10.1038/nature04302.

Káradóttir, R., Hamilton, N.B., Bakiri, Y., Attwell, D., 2008. Spiking and nonspiking classes of oligodendrocyte precursor glia in CNS white matter. Nat. Neurosci. 11, 450–456, http://dx.doi.org/10.1038/nn2060.

Karschin, A., Wischmeyer, E., Davidson, N., Lester, H.A., 1994. Fast inhibition of inwardly rectifying K+ channels by multiple neurotransmitter receptors in oligodendroglia. Eur. J. Neurosci. 6, 1756–1764, http://dx.doi.org/10.1111/j.1460-9568.1994.tb00568.x.

Kastritsis, C.H., McCarthy, K.D., 1993. Oligodendroglial lineage cells express neuroligand receptors. Glia 8, 106–113, http://dx.doi.org/10.1002/glia.440080206.

Kettenmann, H., Von Blankenfeld, G., Trotter, J., 1991. Physiological properties of oligodendrocytes during development. Ann. NY Acad. Sci. 633, 64–77, http://dx.doi.org/10.1111/j.1749-6632.1991.tb15596.x.

Khakh, B.S., Burnstock, G., Kennedy, C., King, B.F., North, R.A., Séguéla, P., Voigt, M., Humphrey, P.P.A., 2001. International union of pharmacology. XXIV. Current status of the nomenclature and properties of P2X receptors and their subunits. Pharmacol. Rev. 53, 107–118.

Khorchid, A., Cui, Q., Molina-Holgado, E., Almazan, G., 2002. Developmental regulation of alpha 1A-adrenoceptor function in rat brain oligodendrocyte cultures. Neuropharmacology 42, 685–696.

Kimoto, S., Okuda, A., Toritsuka, M., Yamauchi, T., Makinodan, M., Okuda, H., Tatsumi, K., Nakamura, Y., Wanaka, A., Kishimoto, T., 2011. Olanzapine stimulates proliferation but inhibits differentiation in rat oligodendrocyte precursor cell cultures. Prog. Neuro-Psychopharmacology. Biol. Psychiatry 35, 1950–1956, http://dx.doi.org/10.1016/j.pnpbp.2011.07.011.

Kirchhoff, F., Kettenmann, H., 1992. GABA triggers a [Ca2+] (i) increase in murine precursor cells of the oligodendrocyte lineage. Eur. J. Neurosci. 4, 1049–1058, http://dx.doi.org/10.1111/j.1460-9568.1992.tb00131.x.

Kirchhoff, F., Mülhardt, C., Pastor, A., Becker, C.-M., Kettenmann, H., 1996. Expression of glycine receptor subunits in glial cells of the rat spinal cord. J. Neurochem. 66, 1383–1390.

Kirischuk, S., Scherer, J., Kettenmann, H., Verkhratsky, A., 1995. Activation of P2-purinoreceptors triggered Ca2+ release from InsP3-sensitive internal stores in mammalian oligodendrocytes. J. Physiol. 483 (Pt 1), 41–57.

Kofuji, P., Ceelen, P., Zahs, K.R., Surbeck, L.W., Lester, H.A., Newman, E.A., 2000. Genetic inactivation of an inwardly rectifying potassium channel (Kir4.1 subunit) in mice: phenotypic impact in retina. J. Neurosci. 20, 5733–5740.

Kressin, K., Kuprijanova, E., Jabs, R., Seifert, G., Steinhäuser, C., 1995. Developmental regulation of Na+ and K+ conductances in glial cells of mouse hippocampal brain slices. Glia 15, 173–187, http://dx.doi.org/10.1002/glia.440150210.

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

Kukley, M., Dietrich, D., 2009. Kainate receptors and signal integration by NG2 glial cells. Neuron Glia Biol. 5, 13–20, http://dx.doi.org/10.1017/S1740925X09990081.

Kukley, M., Kiladze, M., Tognatta, R., Hans, M., Swandulla, D., Schramm, J., Dietrich, D., 2008. Glial cells are born with synapses. FASEB J. 22, 2957–2969, http://dx.doi.org/10.1096/fj.07-090985.

Kuner, T., Schoepfer, R., 1996. Multiple structural elements determine subunit specificity of Mg2+ block in NMDA receptor channels. J. Neurosci. 16, 3549–3558.

Kusumi, I., Boku, S., Takahashi, Y., 2015. Psychopharmacology of atypical antipsychotic drugs: From the receptor binding profile to neuroprotection and neurogenesis. Psychiatry Clin. Neurosci. 69, 243–258, http://dx.doi.org/10.1111/pcn.12242.

Larocca, J.N., Almazan, G., 1997. Acetylcholine agonists stimulate mitogen-activated protein kinase in oligodendrocyte progenitors by muscarinic receptors. J. Neurosci. Res. 50, 743–754, http://dx.doi.org/10.1002/(SICI)1097-4547(19971201)50:5<743::AID-JNR11>3.0.CO;2-2.

Lin, S.-C., Bergles, D.E., 2004. Synaptic signaling between GABAergic interneurons and oligodendrocyte precursor cells in the hippocampus. Nat. Neurosci. 7, 24–32, http://dx.doi.org/10.1038/nn1162.

Lin, S.-C., Bergles, D.E., 2002. Physiological characteristics of NG2-expressing glial cells. J. Neurocytol. 31, 537–549, http://dx.doi.org/10.1023/A:1025799816285.

Lomeli, H., Sprengel, R., Laurie, D.J., Köhr, G., Herb, a, Seeburg, P.H., Wisden, W., 1993. The rat delta-1 and delta-2 subunits extend the excitatory amino acid receptor family. FEBS Lett. 315, 318–322, http://dx.doi.org/10.1016/0014-5793(93)81186-4.

Lukas, R.J., Changeux, J.-P., Le Novère, N., Albuquerque, E.X., Balfour, D.J., Berg, D.K., Bertrand, D., Chiappinelli, V.A., Clarke, P.B.S., Collins, A.C., Dani, J.A., Grady, S.R., Kellar, K.J., Lindstrom, J.M., Marks, M.J., Quik, M., Taylor, P.W., Wonnacott, S., 1999. International Union of Pharmacology. XX. Current status of the nomenclature for nicotinic acetylcholine receptors and their subunits. Pharmacol. Rev. 51, 397–401.

Lundgaard, I., Luzhynskaya, A., Stockley, J.H., Wang, Z., Evans, K.A., Swire, M., Volbracht, K., Gautier, H.O.B., Franklin, R.J.M., Ffrench-Constant, C., Attwell, D., Káradóttir, R.T., 2013. Neuregulin and BDNF Induce a Switch to NMDA Receptor-Dependent Myelination by Oligodendrocytes. PLoS Biol. 11, e1001743, http://dx.doi.org/10.1371/journal.pbio.1001743.

Luyt, K., Slade, T.P., Dorward, J.J., Durant, C.F., Wu, Y., Shigemoto, R., Mundell, S.J., Váradi, A., Molnár, E., 2007. Developing oligodendrocytes express functional GABAB receptors that stimulate cell proliferation and migration. J. Neurochem. 100, 822–840, http://dx.doi.org/10.1111/j.1471-4159.2006.04255.x.

Luyt, K., Váradi, A., Durant, C.F., Molnár, E., 2006. Oligodendroglial metabotropic glutamate receptors are developmentally regulated and involved in the prevention of apoptosis. J.

Neurochem. 99, 641–656, http://dx.doi.org/10.1111/j.1471-4159.2006.04103.x.

Luyt, K., Varadi, A., Molnár, E., 2003. Functional metabotropic glutamate receptors are expressed in oligodendrocyte progenitor cells. J. Neurochem. 84, 1452–1464, http://dx.doi.org/10.1046/j.1471-4159.2003.01661.x.

Lynch, J.W., 2009. Native glycine receptor subtypes and their physiological roles. Neuropharmacology 56, 303–309, http://dx.doi.org/10.1016/j.neuropharm.2008.07.034.

Makara, J.K., Rappert, A., Matthias, K., Steinhäuser, C., Spät, A., Kettenmann, H., 2003. Astrocytes from mouse brain slices express ClC-2-mediated Cl- currents regulated during development and after injury. Mol. Cell. Neurosci. 23, 521–530, http://dx.doi.org/10.1016/S1044-7431(03)00080-0.

Maldonado, P.P., Vélez-Fort, M., Angulo, M.C., 2011. Is neuronal communication with NG2 cells synaptic or extrasynaptic?. J. Anat. 219, 8–17, http://dx.doi.org/10.1111/j.1469-7580.2011.01350.x.

Maldonado, P.P., Vélez-Fort, M., Levavasseur, F., Angulo, M.C., 2013. Oligodendrocyte precursor cells are accurate sensors of local K+ in mature gray matter. J. Neurosci. 33, 2432–2442, http://dx.doi.org/10.1523/JNEUROSCI.1961-12.2013.

Matsuda, K., Yuzaki, M., 2012. Cbln1 and the delta2 glutamate receptor-An orphan ligand and an orphan receptor find their partners. Cerebellum 11, 78–84, http://dx.doi.org/10.1007/s12311-010-0186-5.

Matyash, V., Kettenmann, H., 2010. Heterogeneity in astrocyte morphology and physiology. Brain Res. Rev. 63, 2–10, http://dx.doi.org/10.1016/j.brainresrev.2009.12.001.

Maxishima, M., Shiga, T., Shutoh, F., Hamada, S., Maeshima, T., Okado, N., 2001. Serotonin 2A receptor-like immunoreactivity is detected in astrocytes but not in oligodendrocytes of rat spinal cord. Brain Res. 889, 270–273, http://dx.doi.org/10.1016/S0006-8993(00)03150-4.

Mei, F., Fancy, S.P.J., Shen, Y.-A.A., Niu, J., Zhao, C., Presley, B., Miao, E., Lee, S., Mayoral, S.R., Redmond, S.A., Etxeberria, A., Xiao, L., Franklin, R.J.M., Green, A., Hauser, S.L., Chan, J.R., 2014. Micropillar arrays as a high-throughput screening platform for therapeutics in multiple sclerosis. Nat. Med. 20, 954–960, http://dx.doi.org/10.1038/nm.3618.

Micu, I., Jiang, Q., Coderre, E., Ridsdale, A., Zhang, L., Woulfe, J., Yin, X., Trapp, B.D., McRory, J.E., Rehak, R., Zamponi, G.W., Wang, W., Stys, P.K., 2006. NMDA receptors mediate calcium accumulation in myelin during chemical ischaemia. Nature 439, 988–992, http://dx.doi.org/10.1038/nature04474.

Monteilh-Zoller, M.K., Hermosura, M.C., Nadler, M.J.S., Scharenberg, A.M., Penner, R., Fleig, A., 2003. TRPM7 provides an ion channel mechanism for cellular entry of trace metal ions. J. Gen. Physiol. 121, 49–60, http://dx.doi.org/10.1085/jgp.20028740.

Nacher, J., McEwen, B.S., 2006. The role of N-methyl-D-asparate receptors in neurogenesis. Hippocampus 16, 267–270, http://dx.doi.org/10.1002/hipo.20160.

Neusch, C., Papadopoulos, N., Müller, M., Maletzki, I., Winter, S.M., Hirrlinger, J., Handschuh, M., Bähr, M., Richter, D.W., Kirchhoff, F., Hülsmann, S., 2006. Lack of the Kir4.1 channel subunit abolishes K+ buffering properties of astrocytes in the ventral respiratory group: impact on extracellular K+ regulation. J. Neurophysiol. 95, 1843–1852, http://dx.doi.org/10.1152/jn.00996.2005.

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.

Niswender, C.M., Conn, P.J., 2010. Metabotropic glutamate receptors: physiology, pharmacology, and disease. Annu. Rev. Pharmacol. Toxicol. 50, 295–322, http://dx.doi.org/10.1146/annurev.pharmtox.011008.145533.

Niu, J., Mei, F., Li, N., Wang, H., Li, X., Kong, J., Xiao, L., 2010. Haloperidol promotes proliferation but inhibits differentiation in rat oligodendrocyte progenitor cell cultures. Biochem. Cell Biol. 88, 611–620, http://dx.doi.org/10.1139/O09-178.

Notomi, T., Shigemoto, R., 2004. Immunohistochemical Localization of Ih Channel Subunits, HCN1-4, in the Rat Brain. J. Comp. Neurol. 471, 241–276, http://dx.doi.org/10.1002/cne.11039.

Nwaobi, S.E., Lin, E., Peramsetty, S.R., Olsen, M.L., 2014. DNA methylation functions as a critical regulator of Kir4.1 expression during CNS development. Glia 62, 411–427, http://dx.doi.org/10.1002/glia.22613.

Olsen, R.W., Sieghart, W., 2008. International Union of Pharmacology. LXX. Subtypes of gamma-aminobutyric acid (A) receptors: classification on the basis of subunit composition, pharmacology, and function. Update. Pharmacol. Rev. 60, 243–260, http://dx.doi.org/10.1124/pr.108.00505.

Othman, T., Yan, H., Rivkees, S.A., 2003. Oligodendrocytes Express Functional A1 Adenosine Receptors That Stimulate Cellular Migration. Glia 44, 166–172, http://dx.doi.org/10.1002/glia.10281.

Paez, P.M., Fulton, D., Colwell, C.S., Campagnoni, A.T., 2009. Voltage-operated Ca(2+) and Na(+) channels in the oligodendrocyte lineage. J. Neurosci. Res. 87, 3259–3266, http://dx.doi.org/10.1002/jnr.21938.

Paez, P.M., Fulton, D., Spreuer, V., Handley, V., Campagnoni, A.T., 2011. Modulation of canonical transient receptor potential channel 1 in the proliferation of oligodendrocyte precursor cells by the golli products of the myelin basic protein gene. J. Neurosci. 31, 3625–3637, http://dx.doi.org/10.1523/JNEUROSCI.4424-10.2011.

Pankratov, Y., Lalo, U., Verkhratsky, A., North, R.A., 2006. Vesicular release of ATP at central synapses. Pflug. Arch. Eur. J. Physiol. 452, 589–597, http://dx.doi.org/10.1007/s00424-006-0061-x.

Papay, R., Gaivin, R., Jha, A., McCune, D.F., McGrath, J.C., Rodrigo, M.C., Simpson, P.C., Doze, V.A., Perez, D.M., 2006. Localization of the mouse alpha1A-adrenergic receptor (AR) in the brain: alpha1AAR is expressed in neurons, GABAergic interneurons, and NG2 oligodendrocyte progenitors. J. Comp. Neurol. 497, 209–222, http://dx.doi.org/10.1002/cne.20992.

Papay, R., Gaivin, R., McCune, D.F., Rorabaugh, B.R., Macklin, W.B., McGrath, J.C., Perez, D.M., 2004. Mouse α1B-adrenergic receptor is expressed in neurons and NG2 oligodendrocytes. J. Comp. Neurol. 478, 1–10, http://dx.doi.org/10.1002/cne.20215.

Passlick, S., Grauer, M., Schäfer, C., Jabs, R., Seifert, G., Steinhäuser, C., 2013. Expression of the γ2-subunit distinguishes synaptic and extrasynaptic GABA(A) receptors in NG2 cells of the hippocampus. J. Neurosci. 33, 12030–12040, http://dx.doi.org/10.1523/JNEUROSCI.5562-12.2013.

Pastor, A., Chvátal, A., Syková, 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.

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, http://dx.doi.org/10.1016/0896-6273(94)90277-1.

Puchalski, R.B., Louis, J.C., Brose, N., Traynelis, S.F., Egebjerg, J., Kukekov, V., Wenthold, R.J., Rogers, S.W., Lin, F., Moran, T., Morrison, J.H., Heinemann, S.F., 1994. Selective RNA editing and subunit assembly of native glutamate receptors. Neuron 13, 131–147, http://dx.doi.org/10.1016/0896-6273(94)90464-2.

Ragheb, F., Molina-Holgado, E., Cui, Q.L., Khorchid, A., Liu, H.N., Larocca, J.N., Almazan, G., 2001. Pharmacological and functional characterization of muscarinic receptor subtypes in developing oligodendrocytes. J. Neurochem. 77, 1396–1406, http://dx.doi.org/10.1046/j.1471-4159.2001.00356.x.

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, http://dx.doi.org/10.1002/1098-1136(20010315)33:4<306::AID-GLIA1029>3.0.CO;2-W.

Salter, M.G., Fern, R., 2005. NMDA receptors are expressed in developing oligodendrocyte processes and mediate injury. Nature 438, 1167–1171, http://dx.doi.org/10.1038/nature04301.

Santoro, B., Chen, S., Luthi, A., Pavlidis, P., Shumyatsky, G.P., Tibbs, G.R., Siegelbaum, S.A., 2000. Molecular and functional heterogeneity of hyperpolarization-activated pacemaker channels in the mouse CNS. J. Neurosci. 20, 5264–5275 20/14/5264 [pii].

Sasaki, Y.F., Rothe, T., Premkumar, L.S., Das, S., Cui, J., Talantova, M.V., Wong, H.-K., Gong, X., Chan, S.F., Zhang, D., Nakanishi, N., Sucher, N.J., Lipton, S.A., 2002. Characterization and comparison of the NR3A subunit of the NMDA receptor in recombinant systems and primary cortical neurons. J. Neurophysiol. 87, 2052–2063, http://dx.doi.org/10.1152/jn.00531.2001.

Schaumburg, C., O'Hara, B.A., Lane, T.E., Atwood, W.J., 2008. Human embryonic stem cell-derived oligodendrocyte progenitor cells express the serotonin receptor and are susceptible to JC virus infection. J. Virol. 82, 8896–8899, http://dx.doi.org/10.1128/JVI.00406-08.

Schmidt, K., Eulitz, D., Veh, R.W., 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, http://dx.doi.org/10.1016/S0006-8993(99)01938-1.

Seifert, G., Hüttmann, K., Binder, D.K., Hartmann, C., Wyczynski, A., Neusch, C., Steinhäuser, C., 2009. Analysis of astroglial K+ channel expression in the developing hippocampus reveals a predominant role of the Kir4.1 subunit. J. Neurosci. 29, 7474–7488.

Seifert, G., Rehn, L., Weber, M., Steinhäuser, C., 1997a. AMPA receptor subunits expressed by single astrocytes in the juvenile mouse hippocampus. Mol. Brain Res. 47, 286–294, http://dx.doi.org/10.1523/JNEUROSCI.3790-08.2009.

Seifert, G., Zhou, M., Steinhäuser, C., 1997b. Analysis of AMPA receptor properties during postnatal development of mouse hippocampal astrocytes. J. Neurophysiol. 78, 2916–2923, http://dx.doi.org/10.1016/S0169-328X(97)00059-4.

Shah, M.M., 2014. Cortical HCN channels: function, trafficking and plasticity. J. Physiol. 592, 2711–2719, http://dx.doi.org/10.1113/jphysiol.2013.270058.Cortical.

Shelton, M.K., McCarthy, K.D., 2000. Hippocampal astrocytes exhibit Ca2+-elevating muscarinic cholinergic and histaminergic receptors in situ. J. Neurochem. 74, 555–563, http://dx.doi.org/10.1046/j.1471-4159.2000.740555.x.

Shen, J.X., Yakel, J.L., 2012. Functional α7 nicotinic ACh receptors on astrocytes in rat hippocampal CA1 slices. J. Mol. Neurosci. 48, 14–21, http://dx.doi.org/10.1007/s12031-012-9719-3.

Simpson, K.L., Weaver, K.J., de Villers-Sidani, E., Lu, J.Y.-F., Cai, Z., Pang, Y., Rodriguez-Porcel, F., Paul, I.A., Merzenich, M., Lin, R.C.S., 2011. Perinatal antidepressant exposure alters cortical network function in rodents. Proc. Natl. Acad. Sci. 108, 18465–18470, http://dx.doi.org/10.1073/pnas.1109353108.

Simpson, P.B., Armstrong, R.C., 1999. Intracellular signals and cytoskeletal elements involved in oligodendrocyte progenitor migration. Glia 26, 22–35, http://dx.doi.org/10.1002/(SICI)1098-1136(199903)26:1<22::AID-GLIA3>3.0.CO;2-M.

Sobko, A., Peretz, A., Attali, B., 1998. Constitutive activation of delayed-rectifier potassium channels by a Src family tyrosine kinase in Schwann cells. EMBO J. 17, 4723–4734, http://dx.doi.org/10.1093/emboj/17.16.4723.

Soliven, B., 2001. Calcium signalling in cells of oligodendroglial lineage. Microsc. Res. Tech. 52, 672–679, http://dx.doi.org/10.1002/jemt.1051.

Sontheimer, H., Trotter, J., Schachner, M., Kettenmann, H., 1989. Channel expression correlates with differentiation stage during the development of oligodendrocytes from their precursor cells in culture. Neuron 2, 1135–1145, http://dx.doi.org/10.1016/0896-6273(89)90180-3.

Spampinato, S.F., Merlo, S., Chisari, M., Nicoletti, F., Sortino, M.A., 2015a. Glial metabotropic glutamate receptor-4 increases maturation and survival of oligodendrocytes. Front. Cell. Neurosci. 8, 462, http://dx.doi.org/10.3389/fncel.2014.00462.

Spampinato, S.F., Merlo, S., Chisari, M., Nicoletti, F., Sortino, M.A., 2015b. Glial metabotropic glutamate receptor-4 increases maturation and survival of oligodendrocytes. Front. Cell. Neurosci. 8, 462, http://dx.doi.org/10.3389/fncel.2014.00462.

Steinhäuser, 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, http://dx.doi.org/10.1111/j.1460-9568.1992.tb00897.x.

Steinhäuser, 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, http://dx.doi.org/10.1002/hipo.450040105.

Stevens, B., Porta, S., Haak, L.L., Gallo, V., Fields, R.D., 2002. Adenosine: A neuron-glial transmitter promoting myelination in the CNS in response to action potentials. Neuron 36, 855–868, http://dx.doi.org/10.1016/S0896-6273(02)01067-X.

Sundelacruz, S., Levin, M., Kaplan, D.L., 2009. Role of membrane potential in the regulation of cell proliferation and differentiation. Stem Cell Rev. Rep. 5, 231–246, http://dx.doi.org/10.1007/s12015-009-9080-2.

Surges, R., Brewster, A.L., Bender, R.A., Beck, H., Feuerstein, T.J., Baram, T.Z., 2006. Regulated expression of HCN channels and cAMP levels shape the properties of the h current in developing rat hippocampus. Eur. J. Neurosci. 24, 94–104, http://dx.doi.org/10.1111/j.1460-9568.2006.04880.x.

Tanaka, Y., Tozuka, Y., Takata, T., Shimazu, N., Matsumura, N., Ohta, A., Hisatsune, T., 2009. Excitatory GABAergic activation of cortical dividing glial cells. Cereb. Cortex 19, 2181–2195, http://dx.doi.org/10.1093/cercor/bhn238.

Tang, X., Taniguchi, K., Kofuji, P., 2009. Heterogeneity of Kir4.1 channel expression in glia revealed by mouse transgenesis. Glia 57, 1706–1715, http://dx.doi.org/10.1002/glia.20882.

Tong, X., Li, X.Y., Zhou, B., Shen, W., Zhang, Z.J., Xu, T., Le, Duan, S., 2009. Ca2+ signaling evoked by activation of Na+ channels and Na+/Ca2+ exchangers is required for GABA-induced NG2 cell migration. J. Cell Biol. 186, 113–128, http://dx.doi.org/10.1083/jcb.200811071.

Traynelis, S.F., Wollmuth, L.P., McBain, C.J., Menniti, F.S., Vance, K.M., Ogden, K.K., Hansen, K.B., Yuan, H., Myers, S.J., Dingledine, R., 2010. Glutamate receptor ion channels: Structure, regulation, and function. Pharmacol. Rev. 62, 405–496, http://dx.doi.org/10.1124/pr.109.002451.

Trimmer, J.S., 1991. Immunological identification and characterization of a delayed rectifier K+ channel polypeptide in rat brain. Neurobiology 88, 10764–10768.

Tsaur, M.L., Chou, C.C., Shih, Y.H., Wang, H.L., 1997. Cloning, expression and CNS distribution of Kv4.3, an A-type K+ channel α subunit. FEBS Lett. 400, 215–220, http://dx.doi.org/10.1016/S0014-5793(96)01388-9.

Vautier, F., Belachew, S., Chittajallu, R., Gallo, V., 2004. Shaker-type potassium channel subunits differentially control oligodendrocyte progenitor proliferation. Glia 48, 337–345, http://dx.doi.org/10.1002/glia.20088.

Vélez-Fort, M., Audinat, E., Angulo, M.C., 2009. Functional α7-containing nicotinic receptors of NG2-expressing cells in the hippocampus. Glia 57, 1104–1114, http://dx.doi.org/10.1002/glia.20834.

Vélez-Fort, M., Maldonado, P.P., Butt, A.M., Audinat, E., Angulo, M.C., 2010. Postnatal switch from synaptic to extrasynaptic

transmission between interneurons and NG2 cells. J. Neurosci. 30, 6921–6929, http://dx.doi.org/10.1523/JNEUROSCI.0238-10.2010.

Vennekens, R., Menigoz, A., Nilius, B., 2012. TRPs in the brain. Rev. Physiol. Biochem. Pharmacol. 163, 27–64, http://dx.doi.org/10.1007/112_2012_8.

Ventimiglia, R., Greene, M.I., Geller, H.M., 1987. Localization of beta-adrenergic receptors on differentiated cells of the central nervous system in culture. Proc. Natl. Acad. Sci. USA 84, 5073–5077.

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.

Wahl-Schott, C., Biel, M., 2009. HCN channels: Structure, cellular regulation and physiological function. Cell. Mol. Life Sci. 66, 470–494, http://dx.doi.org/10.1007/s00018-008-8525-0.

Waly, B., El, Macchi, M., Cayre, M., Durbec, P., 2014. Oligodendrogenesis in the normal and pathological central nervous system. Front. Neurosci. 8, 145, http://dx.doi.org/10.3389/fnins.2014.00145.

Walz, W., 2002. Chloride/anion channels in glial cell membranes. Glia 40, 1–10, http://dx.doi.org/10.1002/glia.10125.

Wang, C., Pralong, W.F., Schulz, M.-F., Rougon, G., Aubry, J.-M., Pagliusi, S., Robert, A., Kiss, J.Z., 1996. Functional N-methyl-D-aspartate receptors in O-2A glial precursor cells: a critical role in regulating polysialic acid-neural cell adhesion molecule expression and cell migration. J. Cell Biol. 135, 1565–1581, http://dx.doi.org/10.1083/jcb.135.6.1565.

Wang, D.D., Krueger, D.D., Bordey, A., 2003. GABA depolarizes neuronal progenitors of the postnatal subventricular zone via GABAA receptor activation. J. Physiol. 550, 785–800, http://dx.doi.org/10.1113/jphysiol.2003.042572.

Wang, H., Xu, H., Niu, J., Mei, F., Li, X., Kong, J., Cai, W., Xiao, L., 2010. Haloperidol activates quiescent oligodendroglia precursor cells in the adult mouse brain. Schizophr. Res. 119, 164–174, http://dx.doi.org/10.1016/j.schres.2010.02.1068.

Wang, W., Gao, X.F., Xiao, L., Xiang, Z.H., He, C., 2011. KV7/KCNQ channels are functionally expressed in oligodendrocyte progenitor cells. PLoS One 6, 1–10, http://dx.doi.org/10.1371/journal.pone.0021792.

Wang, W., Putra, A., Schools, G.P., Ma, B., Chen, H., Kaczmarek, L.K., Barhanin, J., Lesage, F., Zhou, M., 2013. The contribution of TWIK-1 channels to astrocyte K(+) current is limited by retention in intracellular compartments. Front. Cell. Neurosci. 7, 246, http://dx.doi.org/10.3389/fncel.2013.00246.

Weerth, S.H., Holtzclaw, L.A., Russell, J.T., 2007. Signaling proteins in raft-like microdomains are essential for Ca2+ wave propagation in glial cells. Cell Calcium 41, 155–167, http://dx.doi.org/10.1016/j.ceca.2006.06.006.

Weisman, G.A., Woods, L.T., Erb, L., Seye, C.I., 2012. P2Y receptors in the mammalian nervous system: pharmacology, ligands and therapeutic potential. CNS Neurol. Disord. Drug Targets 11, 722–738, http://dx.doi.org/10.2174/187152712803581047.

Williamson, A.V., Compston, D.A.S., Randall, A.D., 1997. Analysis of the ion channel complement of the rat oligodendrocyte progenitor in a commonly studied in vitro preparation. Eur. J. Neurosci. 9, 706–720.

Williamson, A.V., Mellor, J.R., Grant, A.L., Randall, A.D., 1998. Properties of GABA(A) receptors in cultured rat oligodendrocyte progenitor cells. Neuropharmacology 37, 859–873, http://dx.doi.org/10.1016/S0028-3908(98)00016-1.

Wyllie, D.J.A., Mathie, A., Symonds, C.J., Cull-Candy, S.G., 1991. Activation of glutamate receptors and glutamate uptake in identified macroglial cells in rat cerebellar cultures. J. Physiol. 432, 235–258.

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

Yasumura, M., Yoshida, T., Lee, S.J., Uemura, T., Joo, J.Y., Mishina, M., 2012. Glutamate receptor δ1 induces preferentially inhibitory presynaptic differentiation of cortical neurons by interacting with neurexins through cerebellin precursor protein subtypes. J. Neurochem. 121, 705–716, http://dx.doi.org/10.1111/j.1471-4159.2011.07631.x.

Yoo, A.S.J., Krieger, C., Kim, S.U., 1999. Process extension and intracellular Ca2+ in cultured murine oligodendrocytes. Brain Res. 827, 19–27, http://dx.doi.org/10.1016/S0006-8993(99)01282-2.

Yoshioka, A., Bacskai, B., Pleasure, D., 1996. Pathophysiology of oligodendroglial excitotoxicity. J. Neurosci. Res. 46, 427–437, http://dx.doi.org/10.1002/(SICI)1097-4547(19961115)46:4<427::AID-JNR4>3.0.CO;2-I.

Yuan, X., Eisen, A.M., 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.

Zhang, Y., Chen, K., Sloan, S.A., Bennett, M.L., Scholze, A.R., O'Keeffe, S., Phatnani, H.P., Guarnieri, P., Caneda, C., Ruderisch, N., Deng, S., Liddelow, S.A., Zhang, C., Daneman, R., Maniatis, T., Barres, B.A., Wu, J.Q., 2014. An RNA-sequencing transcriptome and splicing database of glia, neurons, and vascular cells of the cerebral cortex. J. Neurosci. 34, 11929–11947, http://dx.doi.org/10.1523/JNEUROSCI.1860-14.2014.

Zhou, M., Schools, G.P., Kimelberg, H.K., 2006. Development of GLAST(+) astrocytes and NG2(+) glia in rat hippocampus CA1: mature astrocytes are electrophysiologically passive. J. Neurophysiol. 95, 134–143, http://dx.doi.org/10.1152/jn.00570.2005.

Zhou, M., Xu, G., Xie, M., Zhang, X., Schools, G.P., Ma, L., Kimelberg, H.K., Chen, H., 2009. TWIK-1 and TREK-1 are potassium channels contributing significantly to astrocyte passive conductance in rat hippocampal slices. J. Neurosci. 29, 8551–8564, http://dx.doi.org/10.1523/JNEUROSCI.5784-08.2009.

Ziskin, J.L., Nishiyama, A., Rubio, M., Fukaya, M., Bergles, D.E., 2007. Vesicular release of glutamate from unmyelinated axons in white matter. Nat. Neurosci. 10, 321–330, http://dx.doi.org/10.1038/nn1854.

Zonouzi, M., Renzi, M., Farrant, M., Cull-Candy, S.G., 2011. Bidirectional plasticity of calcium-permeable AMPA receptors in oligodendrocyte lineage cells. Nat. Neurosci. 14, 1430–1438, http://dx.doi.org/10.1038/nn.2942.